EMA-amplicon-based sequencing informs risk assessment analysis of water treatment systems

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Title: Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa

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Corresponding Author: Professor Wesaal Khan, Ph.D.

Corresponding Author's Institution: Stellenbosch University

First Author: Brandon Reyneke

Order of Authors: Brandon Reyneke; Thando Ndlovu; Martin B Vincent; Azahara Martínez-García; Maria I Polo-López; Pilar Fernández-Ibáñez; Giuliana Ferrero; Sehaam Khan; Kevin G McGuigan; Wesaal Khan, Ph.D.

Abstract: The efficiency of two large-volume batch solar reactors [Prototype I (140 L) and II (88 L)] in treating rainwater on-site in a local informal settlement and farming community was assessed. Untreated [Tank 1 and Tank 2-(First-flush)] and treated (Prototype I and II) tank water samples were routinely collected from each site and all the measured physico-chemical parameters (e.g. pH and turbidity, amongst others), anions (e.g. sulphate and chloride, amongst others) and cations (e.g. iron and lead, amongst others) were within national and international drinking water guidelines limits. Culture-based analysis indicated that Escherichia coli, total and faecal coliforms, enterococci and heterotrophic bacteria counts exceeded drinking water guideline limits in 61%, 100%, 45%, 24% and 100% of the untreated tank water samples collected from both sites. However, an 8 hour solar exposure treatment for both solar reactors was sufficient to reduce these indicator organisms to within national and international drinking water standards, with the exception of the heterotrophic bacteria which exceeded the drinking water standard limit in 43% of the samples treated with the Prototype I reactor (1 log reduction). Molecular viability analysis subsequently indicated that mean overall reductions of 75% and 74% were obtained for the analysed indicator organisms (E. coli and enterococci spp.) and opportunistic pathogens (Klebsiella spp., Legionella spp., Pseudomonas spp., Salmonella spp. and Cryptosporidium spp. oocysts) in the Prototype I and II solar reactors, respectively. The large-volume batch solar reactor prototypes could thus effectively provide four (88 L Prototype II) to seven (144 L Prototype I) people on a daily basis with the basic water requirement for human activities (20 L). Additionally, a generic Water Safety Plan was developed to aid practitioners in identifying risks and implement remedial actions in this type of installation in order to ensure the safety of the treated water.

Response to Reviewers: To Whom It May Concern:

Please find comments addressing revision recommendations for the article STOTEN-D-19-17763, “Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa”, outlined below. Please note that recommendations by the reviewer will be listed first (bold) followed by the authors response. Similar recommendations made by reviewers will be addressed simultaneously.

Reviewer Two (#2):
Comment 1: The treatment efficiencies of two large-volume batch solar reactors for treating rainwater for house applications were reported. The team has collected field data from two sub-Saharan Africa communities and compared water quality collected using different roof top rain harvesting systems. The physical, chemical properties as well as microbiological quality of the water were evaluated. The manuscript is well written and easy to follow. The work has the potential to guide the practical application in establishing rain harvesting and solar treatment system in low resource communities.
Thank you for the comment.

Comment 2: One interesting results of the study is the significant mismatch of culture-based results and molecular biology-based outcomes. Culture-based assay indicated nearly 3 log-reductions of microbial contaminants for most of the bacterial indicators under solar disinfection. However, molecular biology-based the results suggested no greater than 1-log-removal of the bacterial indicators and pathogens. The potential risks of VBNC organisms in water supply were discussion but should be further emphasized. The conclusion that the solar disinfection of rainwater is effective to treat water that can meet drinking water standard should be presented with caution.
The potential role of VBNC has been clarified and the use of solar disinfection for water treatment has been amended in the conclusion section of the main manuscript as follows:
Lines 585 to 589: “Based on national and international drinking water guidelines (which predominantly employs culture-based analysis), the large-volume batch solar reactor prototypes used in the current study may effectively treat rainwater to within drinking water standards and provide water to the inhabitants of rural areas and urban informal settlements in sub-Saharan Africa.”
Lines 592 to 600: “The discrepancy in the results obtained using culture- and molecular-based analyses highlights the limitations of solely using traditional culture-based analyses to monitor water treatment systems, as an over-estimation of treatment system efficiency may be obtained. Thus, results obtained using molecular-based assays may be more representative of the viable and intact community in the treated water source, and a more accurate indication of the health risk to the end-user may be calculated when this data set is employed in quantitative microbial risk assessment (QMRA). Current research by the WATERSPOUTT research consortium is thus aimed at applying QMRA to monitor the quality of the treated rainwater.”

Reviewer Three (#3):
Comment 1: Graphical abstract - This is an interesting image but might be too large to scale well to a small section on the journal website- please simplify if possible, the main content seems to be the white box with the schematic of the solar treatment apparatus
Reviewer Five (#5):
Comment 2: Graphical abstract - Too dark, better to change the background.

Based on the reviewer recommendations, the graphical abstract has been simplified and the background illustration has been removed.

Comment 2: General - The reviewed manuscript is very comprehensive and addresses the important topic of treating small-scale harvested rainwater using solar disinfection to meet drinking water needs in Cape Town, South Africa. This topic is of interest to readers of STOTEN and the manuscript reads clearly. Importantly, the authors incorporated information about the water usage and held workshops with residents to improve their understanding of issues related to rainwater safety, and developed a water safety plan template. They should be commended for this effort. Supplemental information was very detailed and comprehensive.
Thank you for the comment.

Comment 3: Abstract - Line 29 specify which anions and cations
During the chemical analyses, six anions (i.e. sulphate, chloride, nitrite, nitrate, phosphate and fluoride) and 25 cations (i.e. aluminium, antimony, arsenic, boron, barium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, phosphorus, potassium, selenium, silicon, sodium, strontium, vanadium and zinc) were monitored. Due to the journal "Abstract" word limit, all the anions and cations cannot be specified within this section.
The abstract has however been amended as follows:
Lines 28 to 31: "…all the measured physico-chemical parameters (e.g. pH and turbidity, amongst others), anions (e.g. sulphate and chloride, amongst others) and cations (e.g. iron and lead, amongst others) were within national and international drinking water guidelines limits."

All the tested anions and cations, their respective concentrations and concentration limits stipulated by the reference drinking water guidelines [i.e. South African National Standards 241 (South African Bureau of Standards, 2005); Department of Water Affairs and Forestry (1996), Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011) and World Health Organization (WHO) (2017)] are outlined in Table A.3 of the supplementary information and referred to in Lines 178, 181, 313, 316 and 341 of the main manuscript.

Comment 4: Abstract - Line 39-40: indicate of spp. or specific species
The quantitative polymerase chain reaction (qPCR) assays used for the quantification of the target organisms in the current study, were genus specific with the exception of the qPCR assay used for Escherichia coli (E. coli). The term "spp." has been inserted in the abstract to clarify this as follows:
Lines 39 to 41: "…analysed indicator organisms (E. coli and enterococci spp.) and opportunistic pathogens (Klebsiella spp., Legionella spp., Pseudomonas spp., Salmonella spp. and Cryptosporidium spp. oocysts) in the…"

Comment 5: Introduction - Line 50: The Global Risks Report is produced by the World Economic Forum- please edit citation accordingly. Please give the scale of the rankings (1-10, where 1 is low and 10 is high?)
Thank you for the comment. The citation for the Global Risks Report has been amended in-text and in the reference list as follows:
Line 52: "…(World Economic Forum, 2019)."
Line 740 to 741: "World Economic Forum., 2019. The Global Risks Report 2019 14th Edition. Available: http://www3.weforum.org/docs/WEF_Global_Risks_Report_2019.pdf. [2019, February 10]."

The Global Risks Report classifies the top 10 risks based on “likelihood of occurring” and “impact”. Additionally, a scale of 1 to 5 was used by respondents to classify both the “likelihood” (1: a risk that is very unlikely to occur to 5: a risk that is very likely to occur) and “impact” (1: minimal impact, 2: minor impact, 3: moderate impact, 4: severe impact and 5: catastrophic impact) of each global risk. The risk posed by “water crises” ranked 9th (out of 10) in terms of likelihood and 4th (out of 10) in terms of impact. The terms “rating of 9” and “rating of 4” have thus been replaced by the terms “9th overall” and “4th overall” in the manuscript as follows:
Lines 50 to 52: “The Global Risks Report released for 2019 listed water crises as one of the top ten risks in terms of likelihood (9th overall; very likely to occur) and impact (4th overall; severe impact) (World Economic Forum, 2019).”

Comment 6: Introduction - Line 57 replace "exploited" with another word like "underutilized" since exploited has negative connotations
The term “under-exploited” has been replaced with the term “underutilised” as follows:
Line 57: “…rainwater is considered an underutilised water source in sub-Saharan Africa…”

Comment 7: Introduction - Line 64-65 these pathogens are not only fecal-associated; also originating from biofilms or indigenously present? Please indicate.
We are in agreement that not all of the listed microbial contaminants, namely Legionella, Klebsiella, Pseudomonas and Cryptosporidium, within harvested rainwater are only associated with faecal matter. As indicated in Line 62, these microbial contaminants may also originate from organic debris being washed into the rainwater harvesting tank during a rain event. However, Bauer et al. (2003) and Kaushik et al. (2012) reported on the presence of E. coli, Pseudomonas aeruginosa and Klebsiella pneumoniae in freshly captured rainwater, indicating that these organisms may be indigenously present within this water source. Woo et al. (2013) and Wei et al. (2016) then reported on the detection of Legionella spp., Pseudomonas spp., Salmonella spp., and Staphylococcus spp. in bioaerosol particles, which may elucidate why these organisms may also be detected in “fresh” rainwater.
The sentence regarding the origin of the microbial contaminants has been amended as follows:
Line 61 to 65: “While the chemical pollutants have not been directly associated with the incidence of disease, organic debris, faecal matter from animals that have access to the catchment surface and bioaerosol particles, have been identified as the primary sources of microbial contaminants such as Legionella, Klebsiella, Pseudomonas and Cryptosporidium (Hamilton et al., 2019).”

• Bauer, H., Giebl, H., Hitzenberger, R., Kasper-Giebl, A., Reischl, G., Zibuschka, F., Puxbaum, H., 2003. Airborne bacteria as cloud condensation nuclei. J. Geophys. Res. 108, 4658-4665. https://doi.org/10.3390/atmos10120786.
• Kaushik, R., Balasubramanian, R., De La Cruz, A.A., 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. Appl. Environ. Microbiol. 78, 2813-2818. https://doi.org/10.1128/AEM.07695-11.
• Wei, K., Zou, Z., Zheng, Y., Li, J., Shen, F., Wu, C., Wu, Y., Hu, M., Yao, M., 2016. Ambient bioaerosol particle dynamics observed during

haze and sunny days in Beijing. Sci. Total Environ. 550, 751-759. https://doi.org/10.1016/j.scitotenv.2016.01.137.
• Woo, A.C., Manreetpal, S.B., Chan, Y., Lau, M.C.Y., Leung, F.C.C., Scott, J.A., Vrijmoed, L.L.P., Zawar-Reza, P., Pointing, S.B., 2013. Temporal variation in airborne microbial populations and microbially-derived allergens in a tropical urban landscape. Atmos. Environ. 74, 291-300. https://doi.org/10.1016/j.atmosenv.2013.03.047.

Comment 8: Introduction - Line 74-75 does the PET container contain phthalates? These are endocrine disruptors, is there any concern for leaching of these materials from the plastic?
As PET was not used in the construction of the large-volume batch solar reactor prototypes, the potential leaching of plasticisers from PET was not discussed in the current article. However, members of the WATERSPOUTT research consortium are currently assessing the potential leaching of endocrine disruptors from poly(methyl methacrylate) (PMMA) (also known as plexiglass), which was used in the current study for the construction of the large-volume batch solar reactor prototypes. The potential leaching of endocrine disruptors from PMMA has been included in the "Results" section as follows:
Lines 548 to 550: "The potential degradation (leaching) of the PMMA reactor tubing is however, being investigated by members of the WATERSPOUTT research consortium."

Comment 9: Introduction - Line 89, 98, 106 "unpublished results" does not appear in the reference list or supplemental documents, please cite this in reference list as unpublished manuscript, personal communication, or include description in SI
The in-text references to "unpublished results", namely Martínez-García et al. (Unpublished results A) and Martínez-García et al. (Unpublished results B) are included in the reference list as follows:
Lines 676 to 678: "Martínez-García, A., Domingos, M., Canela, M.C., Oller, I., Vincent, M., Fernández-Ibáñez, P., Polo-López, M.I., (Unpublished results A). Comparative assessment of CPC and V-trough solar reactors for the disinfection of rainwater."

Lines 679 to 681: "Martínez García, A., Polo-López, M.I., Oller, I., Vincent, M., Fernández-Ibáñez, P., (Unpublished results B). Novel large-scale solar reactor for disinfection of rainwater: assessment of a consortium of bacteria and phages."

Comment 10: Introduction - Line 112, 116: Is Salmonella considered as a frank or opportunistic pathogen? Cite reference justifying consideration as opportunistic pathogen if categorizing as such
While almost all strains of Salmonella are considered pathogenic (due to their ability to invade, replicate and survive in human hosts), generally, children (< 5 years old), the elderly and immunocompromised patients are more susceptible to Salmonella infection in comparison to healthy individuals (Eng et al. 2015). Research has also indicated that certain strains lack the ability to persist in the host cell (which is crucial for pathogenesis) and are thus non-virulent (Bakowski et al. 2008). Moreover, certain serotypes are host-specific and can only reside in one or a few animal species [e.g. Salmonella enterica serotype Dublin (cattle) and Salmonella enterica serotype Choleraesuis (swine)] (WHO, 2018). Due to the potential of the culture-based and molecular-based assays to detect a wide range of species in the Salmonella genus (pathogenic and opportunistic pathogenic spp.; human vs non-human specific), the term "opportunistic pathogens" was used in the current

manuscript when referring to the target organisms (e.g. Klebsiella spp., Legionella spp., Pseudomonas spp. and Salmonella spp.).

References justifying the classification of the respective target organisms as opportunistic pathogens have been added as follows:
Lines 119 to 121: "…and opportunistic pathogens (Klebsiella spp., Legionella spp., Pseudomonas spp., and Salmonella spp.) (Fields et al., 2002; Eng et al., 2015; Clements et al., 2019; Strauss et al., 2019), while propidium…"

All added references were already available in the reference list, with the exception of Eng et al. (2015), which has subsequently been added (Lines 639 to 641).

- Bakowski, M.A., Braun, V., Brumell, J.H., 2008. Salmonella containing vacuoles: directing traffic and nesting to grow. Traffic. 9, 2022-2031. https://doi.org/10.1111/j.1600-0854.2008.00827.x.
- Eng, S-K., Pusparajah, P., Ab Mutalib, N-S., Ser, H-L., Chan, K-G., Lee, L-H., 2015. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Front. Life Sci. 8, 284-293. https://doi.org/10.1080/21553769.2015.1051243.
- World Health Organization (WHO)., 2018. Salmonella (non-typhoidal). Available: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal). [2020, January 20].

Comment 11: Introduction - Line 138 did the first flush diverter (Superhead rainwater filter) also include a filtration unit and if so what kind/pore size? Or just a diversion of first flush volume or a mesh screen?
Reviewer Five (#5):
Comment 8: Which type of first flush diversion system was installed? Smiler one in the attachment?
A Superhead® rainwater filter was installed at the site. The system contains a traditional first-flush diverter with a mesh leaf screen opening. As water flows into the unit, it is automatically diverted through the one-way filter into the flush pipe. As soon as the flush pipe is full, the clean water is diverted into the rainwater tank through an insect screen (stopping any insects or floating debris from getting into the water tank). The information has been amended as follows:
Line 142 to 143: "…, a first-flush (FF) diverter with built-in leaf and insect screens (Superhead® rainwater filter) was installed to redirect the initial roof run-off during a rain event (Fig. 1.B)."

Comment 12: Methods - Line 169-170 give a brief description of the chemical analysis process/ instrument type(s) used and which cations and anions were monitored for. Why were only a subset of samples monitored for anions and turbidity?
Representative samples were analysed for anions and turbidity as previous research conducted by members of our research group indicated that anion concentrations in rainwater collected from the region (Stellenbosch), adhered to drinking water standards (Dobrowsky et al., 2015; Reyneke et al., 2016; 2018; Strauss et al., 2016; 2018). Similarly, the rainwater samples were also found to have low levels of turbidity [<1.00 Nephelometric Turbidity Units (NTU)], which adhered to drinking water standards (Strauss et al. 2016; 2018).

A brief description of the chemical analysis process and an explanation of why representative samples were analysed for anion and turbidity concentrations has been added as follows:
Lines 175 to 183: "Briefly, for cation analysis, 50 mL Falcon™ high-clarity polypropylene tubes (Corning Life Sciences, USA) and polyethylene caps were pre-treated with 1% nitric acid before sample collection. Following sample collection, the concentration of 25 cations (outlined in Table A.3 of the supplementary information) were determined after acidification (1% ultrapure nitric acid) using inductively coupled plasma mass spectrometry (Agilent 7700 ICP-MS) by the Central Analytical Facility (CAF) at Stellenbosch University. One litre water samples were collected for anion and turbidity analyses (outlined in Table A.3 of the supplementary information) and processed by Bemlab Laboratories (Cape Town, South Africa) using a Thermo Scientific Gallery™ Automated Photometric Analyser."

Lines 185 to 191: "Representative samples were analysed for anions and turbidity as previous research conducted by members of our research group indicated that anion concentrations in rainwater collected from the region (Stellenbosch), adhered to drinking water standards (Dobrowsky et al., 2015; Reyneke et al., 2016; 2018; Strauss et al., 2016; 2018). Similarly, the rainwater samples were also found to have low levels of turbidity [<1.00 Nephelometric Turbidity Units (NTU)] which adhered to drinking water standards (Strauss et al. 2016; 2018)."

- Dobrowsky, P.H., Carstens, M., De Villiers, J., Cloete, T.E., Khan, W., 2015. Efficiency of a closed-coupled solar pasteurization system in treating roof harvested rainwater. Sci. Total Environ. 536, 206-214. https://doi.org/10.1016/j.scitotenv.2015.06.126.
- Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W., 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing Legionella contamination of roof-harvested rainwater. Sci. Total Environ. 553, 662-670. https://doi.org/10.1016/j.scitotenv.2016.02.108.
- Reyneke, B., Cloete, T.E., Khan, S., Khan, W., 2018. Rainwater harvesting solar pasteurization treatment systems for the provision of an alternative water source in peri-urban informal settlements. Environ. Sci: Water Res. Technol. 4, 291-302. https://doi.org/10.1039/c7ew00392g.
- Strauss A., Dobrowsky P.H., Ndlovu T., Reyneke B., Khan W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. BMC Microbiol. 16, 289. https://doi.org/10.1186/s12866-016-0909-y.
- Strauss A., Reyneke B., Waso M., Khan W., 2018. Compound parabolic collector solar disinfection system for the treatment of harvested rainwater. Environ. Sci.: Water Res. Technol. 4, 976-991. https://doi.org/10.1039/C8EW00152A.

Comment 13: Methods - Line 176 briefly describe filtration- what effective volume was analyzed for the culture samples?
Comment 14: Methods - Line 185-186 give media and conditions for Klebsiella, Pseudomonas, Salmonella spp.
Detailed information regarding the culture-based analyses for the respective indicator organisms [E. coli, total coliforms, faecal coliforms, enterococci and heterotrophic bacteria (HPC)] and opportunistic pathogens (Klebsiella spp., Pseudomonas spp. and Salmonella spp.) has been added as follows:
Filtration for E. coli and total coliforms - Lines 196 to 201: "Briefly, a total volume of 100 mL (undiluted, 10-1 and 10-2) was filtered through

a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm2 at 0.7 bar (70 kPa). The filters were then placed onto Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) and were incubated at 35 ± 2 °C for 18 - 24 hrs."

Enterococci, faecal coliforms and HPC – Lines 201 to 209: "In order to enumerate enterococci, 100 µL of an undiluted sample was spread plated onto Slanetz and Bartley Agar (Oxoid), with the plates incubated for 44 – 48 hrs at 36 ± 2 °C (Strauss et al., 2016). In order to enumerate faecal coliforms (FC), 100 µL of an undiluted sample was spread plated onto m-FC Agar (Biolab, Merck, Wadeville, South Africa), with the plates incubated for 44 – 48 hrs at 35 ± 2 °C (Strauss et al., 2016). For the enumeration of the heterotrophic plate count/bacteria (HPC), a serial dilution (10-1–10-3) was prepared for each sample and by use of the spread plate method 100 µL of an undiluted sample and each dilution (10-1–10-3) was plated onto Luria Bertani (LB) agar (Biolab), with the plates incubated at 37 °C for up to four days."

Klebsiella spp., Pseudomonas spp. and Salmonella spp. – Lines 214 to 219: "Additionally, Klebsiella spp. (HiCromeTM Klebsiella Selective Agar; Sigma-Aldrich, St Louis, MO), Pseudomonas spp. (Pseudomonas Isolation Agar; Sigma-Aldrich) and Salmonella spp. (Salmonella-Shigella Agar; Oxoid) were enumerated as outlined in Clements et al. (2019) by spread plating 100 µL of an undiluted sample onto the respective media and incubating the plates at 37 °C for 18 to 24 hours."

Comment 15: Methods - Line 200 indicate Cryptosporidium species (or spp.) analyzed. Why only quantify in a subset? Also indicate whether spp. or a particular species in SI table A.1
The primer set for the detection and quantification of Cryptosporidium oocysts targeted the general Cryptosporidium oocyst wall protein. Cryptosporidium spp. oocysts would thus be detected and quantified. The term "Cryptosporidium oocysts" has been replaced by "Cryptosporidium spp. oocysts" throughout the manuscript (Lines 41, 122, 233, 236, 453, 458, 481 and 591) and supplementary information [Table A1, Figure A8 (G)].

Unfortunately as tank water concentration methods were optimised for the EMA analysis, an insufficient volume of water was available for sampling #1 to #8 for the additional tank water concentration and PMA treatment required for Cryptosporidium spp. oocyst detection and quantification. The following information has been added to the manuscript:
Lines 234 to 236: "…(an insufficient volume of water was available for #1 to #8 for the additional tank water concentration and PMA treatment required for Cryptosporidium spp. oocyst detection and quantification)."

Comment 16: Methods - Line 246 paired t-test has underlying assumption of normality of the differences in the variables- please verify this was checked with Wilcoxon rank sum or another test and/or that the data met the assumptions of the parametric t-test.
Thank you for the comment. The information has been amended as follows:
Lines 277 to 285: "Statistical analyses were conducted utilising either RStudio (version 1.0.153) or Minitab19. Shapiro-Wilk tests were performed in order to determine whether the data was evenly or non-evenly distributed. Overall differences in sample composition between site 1 and site 2 and the untreated (Tank 1 and Tank 2) and solar reactor treated (Prototype I and II) tank water samples was then determined by evaluating

all measured physico-chemical, chemical and microbial parameters using either the parametric paired t-test or the non-parametric Wilcoxon test (significant when $p < 0.05$). Principle component analysis (PCA) was then used to visualise the correlations between the measured cations at both sites and identify which cations primarily influenced the sample composition at each site.”

Comment 17: Methods - Appendix 2 part 2 Hazards and hazardous events identification- also animals themselves could get caught in tanks, also for storage tank microbial contamination from buildup of biofilms, scale, algal growth, etc.
The following information has been amended in the Hazards and hazardous events identification section:
Rainwater Storage Tank:
“Microbial and physical [organic matter/plant debris, insects, small animals (rodents, lizards etc.)] contamination enters the storage tank due to a missing or inadequate (e.g. damaged, cracked, leaking, no vermin/insect cover) overflow pipe.”
“Microbial and chemical contamination due to the build-up of biofilms or formation of a sediment layer in the bottom of the tank.”

Comment 18: Results & Discussion - Line 418-419 average reduction in opportunistic pathogens- which pathogens did this include? Is Crypto included in this number?
The reported 74.43% reduction includes all the monitored organisms using EMA-qPCR and PMA-qPCR analysis. The sentence has been amended and the organism names (in brackets) have been added as follows:
Lines 456 to 458: “For the monitored indicator organisms and opportunistic pathogens, EMA-qPCR (E. coli, enterococci, Klebsiella spp., Legionella spp., Pseudomonas spp., and Salmonella spp.) and PMA-qPCR (Cryptosporidium spp. oocysts) analysis…”

Comment 19: Results & Discussion - Line 423-425 this also indicates reason for caution that water meeting the guidelines is safe as this is a weakness of the FIB-regulatory paradigm
We are in agreement and the following information has been added to the manuscript to highlight the limitations of assessing water quality using only indicator bacteria:
Lines 474 to 480: “Thus while the use of indicator bacteria (culture-based analysis) has become routine when monitoring water quality, it should be noted that there is a poor correlation between the presence of faecal indicators and potential pathogenic bacteria (Ahmed et al., 2008). Monitoring for the removal of potentially pathogenic microorganisms which may have entered a VBNC state following water treatment is thus essential as these VBNC bacteria still pose a health risk as they are potentially infectious (Mansi et al., 2014).”

- Ahmed, W., Huygens, F., Goonetilleke, A., Gardner, T., 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. Appl. Environ. Microbiol. 74, 5490-5496. https://doi.org/10.1128/AEM.00331-08.
- Mansi, A., Amori, I., Marchesi, I., Marcelloni, A.M., Proietto, A.R., Ferranti, G., Magini, V., Valeriani, F., Borella, P., 2014. Legionella spp. survival after different disinfection procedures: Comparison between conventional culture, qPCR and EMA-qPCR. Microchem. J. 112, 65-69. https://doi.org/10.1016/j.microc.2013.09.017.

Comment 20: Results & Discussion - Line 428 how much regrowth was there after 24 h? It would be useful to have measurements of the molecular markers for regrowth as well to see if this trend is consistent by method. Looking at typical household water needs/ usage rates in this area, would the entire volume treated be used within 24 h?
The volume of water that was stored to monitor microbial regrowth was insufficient to conduct molecular-based analysis on the sample. However, it was hypothesised in the current study that the discrepancies observed between the culture-based and molecular-based analyses may be attributed to the presence of VBNC. These VBNC cells may then regain their ability to be cultured under favourable conditions or once the cells have initiated DNA repair mechanisms. The mean regrowth (CFU/100 mL) observed in the samples has been included in the manuscript as follows:
Lines 432 to 435: "The treated water collected from the large-volume batch solar reactor prototypes could however, only be stored for a maximum of 24 hours, as microbial regrowth occurred after this point (2.0 × 103 CFU/100 mL to 1.80 × 104 CFU/100 mL detected after 24 hours)."

Based on the minimum essential water requirement for health and hygiene of 20 L per person per day (WHO, 2013), a typical household (4 people) could use the entire volume of treated water produced by the Prototype II solar reactor (88 L), while a household of 7 people could use the volume of water produced by the Prototype I solar reactor (140 L). However, the systems that were installed aimed to serve as an alternative water source to multiple households within the community and thus the entire volume of treated water would be used on a daily basis.

• World Health Organization (WHO)., 2013. How much water is needed in emergencies. Technical notes on drinking-water, sanitation and hygiene in emergencies. Available: https://www.who.int/water_sanitation_health/publications/2011/WHO_TN_09_How_much_water_is_needed.pdf?ua=1. [2020, January 20].

Comment 21: Results & Discussion - Line 488-500 any information gathered on the efficacy of the community education program? It would be interesting to gather some information on this in the future.
Follow-up workshops are currently being conducted with participating community members from both sites, which are aimed at the development of educational material through the implementation of co-design principles and are also aimed at assessing issues based on water governance, gender-based roles related to water and water security, amongst others. As this research is ongoing and part of the larger collaborative Social Science work package of the WATERSPOUTT project, this information was not included in the current manuscript which focused on assessing the microbial and chemical quality of the water produced by the large-volume batch solar reactor prototypes.

Comment 22: Results & Discussion - Is there a "standardized" template or list of required components for a Water Safety plan from WHO or any other organization? Or did the authors develop this completely?
A recommended template for a Water Safety Plan (WSP) and required components is outlined by the WHO (2004) and was summarised in Lines 257 to 275. These main components include: (1) A simplified description of the technology, (2) Hazards and hazardous events identification and risk assessment, (3) Tools for operational monitoring and (4) Management programmes. Using this information, the WSP was compiled (Appendix B and Appendix C). However, as indicated in Appendix B, the risk assessment matrix (3X3 semi-quantitative matrix) was obtained from WHO (2009), while

the checklist (outlined in the "Tools for Operational Monitoring" section) was obtained from the WHO (2018).

• World Health Organization (WHO)., 2004. Guidelines for drinking-water quality. Rev. 3rd ed. World Health Organization. Geneva, Switzerland: WHO Press. ISBN: 92-4-154638-7.
• World Health Organization (WHO)., 2009. Water Safety Plans. Managing drinking-water quality from catchment to consumer. By Bartram, J., Corrales, L., Davison, A., Deere, D., Drury, D., Gordon, B., Howard, G., Rinehold, A., Stevens, M. WHO, Geneva, ISBN 978 92 4 156263 8. Available: https://apps.who.int/iris/bitstream/handle/10665/75141/9789241562638_eng.pdf?sequence=1. [2020, January 20].
• World Health Organization (WHO)., 2018. Draft Management Advice Sheet. Rainwater collection and storage. Available: https://www.who.int/water_sanitation_health/sanitation-waste/sanitation/revision-of-who-sanitary-inspection-forms/en/ [2020, January 20].

Comment 23: Results & Discussion - Based on the description in the appendix, there are many animals in the area of the solar reactors and opportunities for generation of dust, additional debris entering the SODIS reactor. Did the authors notice any substantial differences between any previous bench-scale work and the current measurements taken?
As outlined in Lines 402 to 424, results from the current study indicated that the preliminary pilot-scale analyses of the large-volume batch solar reactor prototypes (Martínez García et al., Unpublished results A) may have overestimated the treatment efficiency of the systems as compared to the results obtained in the current study, where environmental field trial samples were analysed. It was hypothesised that the presence of more resilient environmental strains (of the target organisms) may have contributed to this observation.

• Martínez García, A., Polo-López, M.I., Oller, I., Vincent, M., Fernández-Ibáñez, P., (Unpublished results B). Novel large-scale solar reactor for disinfection of rainwater: assessment of a consortium of bacteria and phages.

Comment 24: Results & Discussion - Could metal ions from the roofing material interfere with the disinfection process? Are metals something that should be measured to make sure they aren't increased during heat treatment?
The concentration of metals should definitely be monitored when assessing water quality and water treatment systems, specifically during heat treatment, as long-term exposure to increased metal concentrations may pose a health risk to the end-user (Martin and Griswold, 2009). While investigating the efficiency of solar pasteurization systems to treat roof-harvested rainwater, members of our research group reported on the leaching of metals (e.g. iron, aluminium and copper amongst others) from the stainless steel storage tank of a solar pasteurization system (Dobrowsky et al., 2015; Reyneke et al., 2016). As the measured concentrations exceeded the limits stipulated by various drinking water guidelines, it was recommended that the storage tank of the solar pasteurization systems be constructed from a high-density polyethylene. Subsequent studies (Strauss et al., 2016; Reyneke et al., 2018) using a new solar pasteurization system (with a high-density polyethylene storage tank) indicated that decreased leaching of metals occurred inside the new

systems; however, as metal pipe connectors were still used in the new solar pasteurization system, leaching of copper and nickel occurred.

Based on these results, the use of metal components in the design of the large-volume batch solar reactor prototypes in the current study was limited to the system frame, with no metal components exposed to the rainwater during treatment. This was confirmed as no significant difference in cation concentration was observed between the untreated and treated rainwater samples at each site (Table A3). The observed cation concentrations recorded in the untreated and treated rainwater samples can thus be attributed to metals (e.g. Zinc) leaching from the metal roofing material during the harvesting process. It has been reported that the presence of metal oxides (eg. ZnO and Fe_2O_3) in the water can contribute to the generation of reactive oxygen species and thus increase SODIS treatment efficiency (Byrne et al., 2011). As the measured cation concentrations were within the drinking water guidelines, the potential leaching of metal components during water treatment was not discussed.

- Byrne, J.A., Fernandez-Ibañez, P., Dunlop, P.S.M., Alrousan, D.M.A., Hamilton, J.W.J., 2011. Photocatalytic enhancement for solar disinfection of water: a review. Int. J. Photoenergy. 2011, 798051. https://doi.org/10.1155/2011/798051.
- Dobrowsky, P.H., Carstens, M., De Villiers, J., Cloete, T.E., Khan, W., 2015. Efficiency of a closed-coupled solar pasteurization system in treating roof harvested rainwater. Sci. Total Environ. 536, 206-214. https://doi.org/10.1016/j.scitotenv.2015.06.126.
- Martin, S., Griswold, W., 2009. Human health effects of heavy metals. Environmental Science and Technology Briefs for Citizens. 15, 1-6.
- Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W., 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing Legionella contamination of roof-harvested rainwater. Sci. Total Environ. 553, 662-670. https://doi.org/10.1016/j.scitotenv.2016.02.108.
- Reyneke, B., Cloete, T.E., Khan, S., Khan, W., 2018. Rainwater harvesting solar pasteurization treatment systems for the provision of an alternative water source in peri-urban informal settlements. Environ. Sci: Water Res. Technol. 4, 291-302. https://doi.org/10.1039/c7ew00392g.
- Strauss A., Dobrowsky P.H., Ndlovu T., Reyneke B., Khan W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. BMC Microbiol. 16, 289. https://doi.org/10.1186/s12866-016-0909-y.

Comment 25: Results & Discussion - A pathogen that was not considered- Naegleria- Fig A.4 you could also discourage nasal rinsing.
Thank you for the comment. We are in agreement that Naegleria may be a protozoan pathogen of concern in harvested rainwater and members of our research group have investigated the presence of specifically Naegleria fowleri in harvested rainwater (Waso et al. 2008) and the inactivation of Naegleria fowleri using a solar pasteurization system (Dobrowsky et al. 2016). In the current study, Cryptosporidium spp. oocysts were monitored as a representative of protozoan contaminants as Cryptosporidium has been recommended as a protozoan water quality indicator (WHO, 2016) and Heaselgrave and Kilvington (2011) reported that Cryptosporidium oocysts (0.14 to 0.32 log reduction) were more UV resistant as compared to Acanthamoeba cysts (2.16 to 3.84 log reduction), Naegleria cysts (3.59 to 3.84 log reduction), Entamoeba cysts (1.90 to 1.92 log reduction),

Giardia cysts (1.94 to 1.96 log reduction) and Ascaris ova (0.56 to 1.42 log reduction).

The risk posed by nasal rinsing is definitely an exposure scenario we will take into consideration in future research as we will be conducting quantitative microbial risk assessment to elucidate the risk associated with using untreated and treated rainwater. The activities outlined on the information poster were selected based on the domestic activities commonly performed within the target communities and were identified based on community surveys [Water Research Commission (WRC) Project Report K5/2368//3, 2016] and personal communication with the target communities.

- Dobrowsky, P.H., Khan, S., Cloete, T.E., Khan, W., 2016. Molecular detection of Acanthamoeba spp., Naegleria fowleri and Vermamoeba (Hartmannella) vermiformis as vectors for Legionella spp. in untreated and solar pasteurized harvested rainwater. Parasite. Vector. 9, 539. https://doi.org/10.1186/s13071-016-1829-2.
- Heaselgrave, W., Kilvington, S., 2011. The efficacy of simulated solar disinfection (SODIS) against Ascaris, Giardia, Acanthamoeba, Naegleria, Entamoeba and Cryptosporidium. Acta Tropica. 119, 138-143. https://doi.org/10.1016/j.actatropica.2011.05.004.
- Waso, M., Dobrowsky, P.H., Hamilton, K.A., Puzon, G., Miller, H., Khan, W., Ahmed, W., 2018. Abundance of Naegleria fowleri in roof-harvested rainwater tank samples from two continents. Environ. Sci. Pollut. Res. Int. 25, 5700-5710. https://doi.org/10.1007/s11356-017-0870-9.
- Water Research Commission (WRC), Project No. K5/2368//3., 2016. Design, Construction and Monitoring of Sustainable Domestic Rainwater Harvesting Treatment Systems in Enkanini Informal Settlement, Stellenbosch. Report to the Water Research Commission, Project No. K5/2368 by Department of Microbiology, Stellenbosch University. Stellenbosch, South Africa.
- World Health Organization (WHO)., 2016. Results of round 1 of the WHO international scheme to evaluate household water treatment technologies. World Health Organization. https://apps.who.int/iris/handle/10665/204284.

Reviewer Five (#5):
Comment 1: Even though it is not very systematic, the results of this field study is important to report. Frequent and extreme weather events make traditional methods inefficient to provide safe drinking water to rural communities and alternative methods, such as the method suggested in this study may be useful. The paper in mainly discusses the microbial water quality and it is better to reflect it in the title, abstract and introduction. Following comments are given to further improve the manuscript.
Thank you for the comment. A comprehensive analysis of the chemical quality of the untreated and treated rainwater was also conducted in the current study (six physico-chemical parameters, 6 anions and 25 cations monitored). These results are summarised in Section 3.1 ("Physico-chemical properties and chemical analysis of the collected tank water samples"), while the measured concentrations and comparison to the respective drinking water quality guidelines are outlined in Table A3.

Comment 3: Better to add a figure with technical specifications. For example, pipe diameter, thickness, lengths, heights, bed angle, orientation, RW tank elevation etc.

Information regarding the component dimensions and the installation of the tanks is summarised in-text (Lines 127 to 143) and outlined in detail in Appendix A of the Supplementary Information ("Description of sampling sites"). However, a schematic diagram (Fig. A3) outlining the measurements of the large-volume batch solar reactor prototypes (and components) has been added to Appendix A of the Supplementary Information. The numbering of the supplementary figures has been updated in Appendix A and main manuscript accordingly.
Reference to the information in the Supplementary Information has been amended in the manuscript as follows:
Lines 143 to 146: "A detailed description of the sampling sites, system installation and schematic diagrams of the large-volume batch solar reactors is outlined in Appendix A, while additional information regarding the working mechanism of the large-volume batch solar reactors is outlined in Appendix B."

Comment 4: Better to show treatment performance with expose to sunlight (no of hours and light intensity).
Information regarding treatment time, the mean UV-A and UB-B irradiance (W/m2) recorded during each solar reactor treatment and temperature (°C) of the collected samples (untreated, treated and total increase in temperature) is outlined in Table A2 (Appendix A, Supplementary Information). Table A2 is referenced in Lines 296, 300 and 304 of the main manuscript.

Comment 5: Better to use rainfall data to justify the concept.
The daily rainfall and ambient temperatures recorded for each day during the 2018/2019 sampling period is outlined in Fig. A6 (Appendix A, Supplementary Information). To clarify this the manuscript has been amended as follows:
Lines 289 to 293: "The daily rainfall and ambient temperatures recorded throughout the 2018/2019 research period as well as the sampling sessions for each site are depicted in Fig. A.6. A total rainfall of 431.4 mm was recorded during July 2018 to September 2018 (high rainfall period), while 183.8 mm was recorded during October 2018 to January 2019 (medium rainfall period). The rainfall then decreased to 146.2 mm during February to April 2019 (low rainfall period)."

Comment 6: Introduction - research question has not been well defined, also use resent publications in similar studies (ex. SENEVIRATHNA, S., RAMZAN, S. & MORGAN, J. 2019. A sustainable and fully automated process to treat stored rainwater to meet drinking water quality guidelines. Process Safety and Environmental Protection, 130, 190-196.).
The current study aimed to assess water treatment systems that could cost-effectively be implemented in developing countries (such as rural areas and urban informal settlements). As such, the introduction aimed to highlight "solar disinfection" (SODIS) as a treatment technology that is currently used within developing countries (Lines 70 to 81) and outline the various limitations that have been identified with using this technique (Lines 81 to 84). These limitations, namely treatment volume and treatment efficiency may then be overcome through the use of SODIS enhancement technologies (Lines 84 to 87). Although we applied the SODIS enhancement technologies to design the large-volume batch solar reactor prototypes and assess their efficiency in controlled pilot-scale studies (Lines 88 to 102), it was necessary to assess the efficiency of the systems on-site in the communities for which these systems had been designed (aim of the current study – Lines 108 to 124). In order to

clarify this, the following information has been added to the introduction:
Lines 103 to 107: "Although the preliminary pilot-scale assessment of the solar reactor prototypes display promise in treating rainwater, it is crucial that these systems be assessed on-site in the target communities, i.e. rural areas and urban informal settlements. This will allow for a more comprehensive indication as to whether these reactors may serve as a sustainable solution in providing communities with a safe alternative water source."

The recommended Senevirathna et al. (2019) reference has been included in-text and in the reference list as follows:
Lines 66 to 70: "Treatment strategies that may be implemented to improve the quality of rainwater include the utilisation of gutter screens or first-flush diverters for the prevention of contaminant entry into the collection tank or post-collection treatment [chemical (e.g. chlorination) and physical treatments (e.g. filtration, solar disinfection (SODIS) and thermal disinfection)] (Hamilton et al., 2019; Senevirathna et al., 2019)."

Lines 713 to 715: "Senevirathna, S.T.M.L.D., Ramzan, S., Morgan, J., 2019. A sustainable and fully automated process to treat stored rainwater to meet drinking water quality guidelines. Process Saf. Environ. 130, 190-196. https://doi.org/10.1016/j.psep.2019.08.005."

Comment 7: Peoples acceptance is important in this type of projects. You better discuss community centred design principles applied in the design and provide evidence to for people's acceptance of this idea.
We are in agreement that the target community members need to be taken into consideration when designing and implementing water treatment systems. Due to the success with which simple SODIS has been implemented in developing countries, the European Union funded WATERSPOUTT (Water Sustainable Point Of Use Treatment Technologies) project (grant agreement no. 688928) aimed to investigate cost-effective and efficient solar-based treatment technologies, with socio-economic sciences and humanities also included in the project (Net4Society, 2018). The development of the solar-based treatment technologies by the WATERSPOUTT research consortium took 2 years and involved dialogue and co-design with the end-users in the target communities as well as the completion of social surveys. The initial prototype of the large-volume batch solar reactors included an aeration system and heating panel to increase treatment efficiency. However, based on the results obtained from these pilot-scale studies and engagement with the community members (assessing their water needs, material availability and economic means) the current prototypes were developed. The results from these shared dialogue workshops could not be included in the current manuscript as the main focus of the current study was to assess the treatment efficiency of the systems on-site in the target communities (focussing on basic sciences), with the results obtained by the socio-economic sciences and humanities partners being prepared for an independent publication. However, an example of the co-design principles followed by our WATERSPOUTT research partners in Malawi for the development of solar-ceramic filtration devices is already publicly available (Buck et al. 2017; Morse et al. 2018).

• Buck, L., Morse, T., Lungu, K., Petney, M., 2017. Interactional co-design and co-production through shared dialogue workshops. In: 2017 International Conference on Engineering and Product Design Education. 7 to 8 September 2017. Oslo and Akerhus University College of Applied

Sciences, Norway. Available: https://bucks.repository.guildhe.ac.uk/17350/1/17350_Buck_L.pdf. [2020, January 20].
• Morse, T., Lungu, K., Luwe, K., Chiwalua, L., Mulwafu, W., Buck, L., Harlow, R., Honor, F., McGuigan, K., 2018. A transdisciplinary co-design and behaviour change approach to introducing SODIS to rural communities in Malawi. In: 2018 Water and Health Conference: Where Science Meets Policy, 29 October to 2 November 2018. University of North Carolina, United States of America. Available: https://strathprints.strath.ac.uk/66270/1/Morse_UNCWHC_2018_A_transdisciplinary_co_design_and_behaviour_change_approach.pdf. [2020, January 20].
• Net4Society., 2018. WATERSPOUTT: a success story in SSH integration. Available: https://www.net4society.eu/files/Net4Society_D_3_3_FINAL_Factsheets_SSH_Integration.pdf. [2020, January 20].

Comment 9: Sampling protocol is very important in this study, which is not well explained in this paper.
In order to clarify the sample collection procedure, the section has been amended as follows:
Lines 152 to 159: "For the microbial and chemical analysis of the water produced by the solar reactor prototypes (Fig. 1), an untreated 10 L sample was collected directly from the RWH tank at each site [hereafter referred to as Tank 1 (Site 1) and Tank 2-FF (Site 2)] on the morning of a sampling event. The respective solar reactor prototypes at each site were then immediately filled with tank water from the RWH tanks and exposed to direct sunlight for 6 hours (sampling sessions 1 to 8) or 8 hours (sampling sessions 9 to 18). Following the completion of the solar exposure, 10 L of each solar treated sample was collected directly from the solar reactors [hereafter referred to as Prototype I (Site 1) and Prototype II (Site 2)]."

Comment 10: Figure 3 - this results seems both tank and prototypes, better to change the figure title.
Thank you for the comment. The figure legend has been amended as follows: "Fig. 3. Box and whiskers plot illustrating the distribution of the intact cells or oocysts/100 mL recorded for each of the target organisms using EMA-qPCR (E. coli, enterococci, Klebsiella spp., Legionella spp., Pseudomonas spp. and Salmonella spp.) and PMA-qPCR (Cryptosporidium spp. oocysts) in the untreated (T1 and T2-FF; solid blue box) and treated (PI and PII; dashed red box) tank water samples collected from (A) site 1 and (B) site 2. The whiskers at the end of each box indicate the minimum and maximum values, while the box is defined by the lower and upper quartiles and the mean value."

Comment 11: The overall procedure seems not systematic, first run the systems with 6 hrs, then increased to 8 hours. If the authors can provide the variation of treatment efficiency with time, it will be more useful to determine optimum time of exposure.
Based on the results obtained by Martínez-García et al. (Unpublished results A) and Martínez-García et al. (Unpublished results B), a 6 hour solar exposure treatment time was identified as sufficient to reduce microbial contaminants in synthetic rainwater. The field-trials were thus initially conducted using a 6 hour solar exposure time. However, based on the results that were obtained [heterotrophic bacteria (HPC) detected in the treated water at levels exceeding the drinking water standards] the treatment time was increased to 8 hours in order to see whether the

increased treatment time would allow for the reduction of HPC to within drinking water standards.

The variation in treatment efficiency based on treatment time is visually represented in Fig. A.8 (Appendix A, Supplementary Information). Additionally, as the total UV exposure will determine treatment efficiency, the mean UV-A and UV-B irradiance (W/m2) recorded during each solar reactor treatment (sampling session 1 to 18) is outlined in Table A2 (Appendix A, Supplementary Information). As outlined in Lines 416 to 422, the increase in treatment time from 6 hours to 8 hours resulted in the mean UV radiation increasing from 20.82 W/m2/h (6 hour treatment) to 24.72 W/m2/h (8 hour treatment). Correspondingly, results indicated that the mean HPC log removal increased from ≥ 1.21 log (6 hour treatment) (Line 411) to ≥ 2.02 log (8 hour treatment) (Line 424).

- Martínez-García, A., Domingos, M., Canela, M.C., Oller, I., Vincent, M., Fernández-Ibáñez, P., Polo-López, M.I., (Unpublished results A). Comparative assessment of CPC and V-trough solar reactors for the disinfection of rainwater.
- Martínez García, A., Polo-López, M.I., Oller, I., Vincent, M., Fernández-Ibáñez, P., (Unpublished results B). Novel large-scale solar reactor for disinfection of rainwater: assessment of a consortium of bacteria and phages.

Comment 12: Line 396-397 do you have results? Otherwise provide the references.
The statement, "The robustness of system components therefore also needs to be taken into consideration when designing water treatment systems for use in rural areas and informal settlements, where replacement components may not be readily available.", was made based on our research groups' experience with assessing water treatment systems on-site in informal settlements (Reyneke et al., 2018). We have noted that the operational sustainability and maintenance of system components may determine whether a treatment technology can successfully be integrated into a target community. Similar observations were made by Mwabi et al. (2011) and McGuigan et al. (2012). The references have been included in-text (Line 553) and were added to the reference list.

- McGuigan, K.G., Conroy, R.M., Mosler, H., Du Preez, M., Ubomba-Jaswa, E., Fernandez-Ibañez, P., 2012. Solar water disinfection (SODIS): a review from bench-top to roof-top. J. Hazard. Mater. 235-236, 29-46. https://doi.org/10.1016/j.jhazmat.2012.07.053.
- Mwabi, J.K., Adeyemo, F.E., Mahlangu, T.O., Mamba, B.B., Brouckaert, B.M., Swartz, C.D., Offringa, G., Mpenyana-Monyatsi, L., Momba, M.N.B., 2011. Household water treatment systems: A solution to the production of safe drinking water by the low-income communities of Southern Africa. Phys. Chem. Earth. 36, 1120-1128. https://doi.org/10.1016/j.pce.2011.07.078.

Comment 13: Operational and maintenance issues encountered are worth to report. Particularly the effect of pipe aging on treatment efficiency, algae growth in the pipes, pipe sedimentation issues, and replacement of parts in prototypes.
As outlined in Lines 545 to 548, monitoring of the operational sustainability of the solar reactor prototypes at both sites indicated that system maintenance was limited to cleaning the surface of the PMMA reactor tubes (prevent dust accumulation that will influence UV

transmittance), with no system components needing replacement during the study period.

Additionally, the installation of the first-flush diverter system at site 2 reduced the entry of organic matter into the rainwater harvesting tank. While at both sites 1 and 2 the outlet tap of the rainwater harvesting tank was located approximately 10 cm from the bottom of the tank. It would therefore be possible for sedimentation to occur inside the rainwater harvesting tank; however, a build-up of organic matter or sedimentation did not occur inside the large-volume batch solar reactor prototypes. Poly(methyl methacrylate) (PMMA) was selected for use in the construction of the solar reactor prototypes as this plastic is considered durable and less likely to scratch. A reduction in the treatment efficiency of the large-volume batch solar reactor prototypes was not observed over the 9-month monitoring period during the current study.

Comment 14: It is worth to indicate the cost of a prototypes ($/prototype), operational cost ($/month) and production cost ($/L) and compare these numbers with other reported rainwater treatment systems. Based on the current large-volume batch solar reactor designs (88 to 140 L treatment volumes) it is estimated that the production cost of treated water will range from US$ 0.01/L to US$ 0.14/L. This estimate was made based on the assumption that the solar reactor prototypes are used eight months of the year (243 days) to treat their full capacity (88 L and 140 L, respectively) and that the systems would have a life expectancy of 8 years. It is however important to note that the current cost estimate may be decreased by replacing the high-grade aluminium framework with a more cost-effective alternative, while large-scale production of the systems will also decrease construction cost.

Information regarding the cost analyses and a summary table (Table A5) comparing different household water treatment technologies has been inserted in Appendix A as follows:
Main manuscript Lines 553 to 555: “A preliminary cost analysis for the solar reactor prototypes has been included in Appendix A, with the cost (US$/L) compared to the costs associated with other household drinking water treatment systems (Table A.5).”

Supplementary Information, Appendix A:
Table A5 Estimated cost analysis of the solar reactor prototypes and comparison to other used household water treatment systems.

Treatment System	Cost (US$/L)	Reference
Large-volume batch solar reactors	0.0 - 0.14	Current Study
Traditional SODIS (2 L)	0.0016	Keogh et al. (2015)
SODIS using a 19L Water Dispenser Container	0.0021	Keogh et al. (2015)
25L SODIS compound parabolic collector	0.002	Ubomba-Jaswa et al. (2010)
Chlorination	0.0007 - 0.1	Sobsey et al. (2008); Shrestha et al. (2018)
Ceramic filtration	0.0018	Shrestha et al. (2018)
Boiling using gas	0.011	Shrestha et al. (2018)
Boiling electricity	0.017	Shrestha et al. (2018)
Reverse osmosis and UV treatment	0.026	Shrestha et al. (2018)

• Keogh, M.B., Castro-Alférez, M., Polo-López, M.I., Fernández Calderero, I., Al-Eryani, Y.A., Joseph-Titus, C., Sawant, B., Dhodapkar, R., Mathur, C., McGuigan, K.G., Fernández-Ibáñez, P., 2015. Capability of

19-L polycarbonate plastic water cooler containers for efficient solar water disinfection (SODIS): Field case studies in India, Bahrain and Spain. Sol. Energy. 116, 1-11. https://doi.org/10.1016/j.solener.2015.03.035.

• Shrestha, K.B., Thapa, B.R., Aihara, Y., Shrestha, S., Bhattarai, A.P., Bista, N., Kazama, F., Shindo, J., 2018. Hidden cost of drinking water treatment and its relation with socioeconomic status in Nepalese urban context. Water. 10, 607. https://doi.org/10.3390/w10050607.

• Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I., McGuigan, K., 2010. Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. J. Chem. Tech. Biotech. 85, 1028-1037. https://doi.org/10.1002/jctb.2398.

Thank you for your time and co-operation.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Cover Letter

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Prof W Khan
Department of Microbiology
Faculty of Science
Tel: +27 21 808 5804
Email: wesaal@sun.ac.za

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8 November 2019

Dear Editor,

We have completed a full research paper titled:
Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa

Authors:
B. Reyneke, T. Ndlovu, M.B. Vincent, A. Martínez-García, M.I. Polo-López, P. Fernández-Ibáñez, G. Ferrero, S. Khan, K.G. McGuigan and W. Khan

The primary aim of the study was to assess the efficiency of the two newly designed WATERSPOUTT large-volume batch solar reactor prototypes (Martínez-García et al. Unpublished results B) for the treatment of RHRW on-site in a local informal settlement (140 L Prototype I) and a rural farming community (88 L Prototype II). A Water Safety Plan (WSP) outlining guidelines for the use of rainwater harvesting combined with solar reactor treatment was also implemented, as this may aid in ensuring the safety of the treated RHRW.

We sincerely hope you will consider this manuscript for review and possible publication.

Thank you for your time and cooperation.

Yours sincerely

Wesaal Khan
Associate Professor (Microbiology)

*Title Page

Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa

B. Reyneke[a], T. Ndlovu[a], M.B. Vincent[b], A. Martínez-García[c], M.I. Polo-López[c], P. Fernández-Ibáñez[c,d], G. Ferrero[e], S. Khan[f], K.G. McGuigan[g] and W. Khan[a*]

[a] Department of Microbiology, Faculty of Science, Stellenbosch University, Private Bag X1, Stellenbosch, 7602, South Africa.

[b] Ecosystem Environmental Services S.A., Sant Andreu de Llavaneres, Barcelona, Spain.

[c] Plataforma Solar de Almeria-CIEMAT, P.O. Box 22, Tabernas, Almería, Spain.

[d] Nanotechnology and Integrated BioEngineering Centre, School of Engineering, University of Ulster, Newtownabbey, Northern Ireland, United Kingdom.

[e] IHE Delft Institute for Water Education, Westvest 7, 2611, AX, Delft, the Netherlands.

[f] Faculty of Health Sciences, University of Johannesburg, PO Box 17011, Doornfontein, 2028, South Africa.

[g] Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland.

Short title: Large-volume batch SODIS treatment of rainwater

Abbreviations[1]

*Corresponding Author: Wesaal Khan; Phone: +27218085804; Fax: +27218085846; Email: wesaal@sun.ac.za

***Responses to Reviewers Comments**

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jou kennisvennoot • your knowledge partner

Prof W Khan
Department of Microbiology
Faculty of Science
Tel: +27 21 808 5804
Fax: +27 21 808 5846
Email: wesaal@sun.ac.za

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24 January 2020

To Whom It May Concern:

Please find comments addressing revision recommendations for the article STOTEN-D-19-17763, "Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa", outlined below. Please note that recommendations by the reviewer will be listed first (bold) followed by the authors response. Similar recommendations made by reviewers will be addressed simultaneously.

Reviewer Two (#2):

Comment 1: The treatment efficiencies of two large-volume batch solar reactors for treating rainwater for house applications were reported. The team has collected field data from two sub-Saharan Africa communities and compared water quality collected using different roof top rain harvesting systems. The physical, chemical properties as well as microbiological quality of the water were evaluated. The manuscript is well written and easy to follow. The work has the potential to guide the practical application in establishing rain harvesting and solar treatment system in low resource communities.
Thank you for the comment.

Comment 2: One interesting results of the study is the significant mismatch of culture-based results and molecular biology-based outcomes. Culture-based assay indicated nearly 3 log-reductions of microbial contaminants for most of the bacterial indicators under solar disinfection. However, molecular biology-based the results suggested no greater than 1-log-removal of the bacterial indicators and pathogens. The potential risks of VBNC organisms in water supply were discussion but should be further emphasized. The conclusion that the solar disinfection of rainwater is effective to treat water that can meet drinking water standard should be presented with caution.
The potential role of VBNC has been clarified and the use of solar disinfection for water treatment has been amended in the conclusion section of the main manuscript as follows:
Lines 585 to 589: "Based on national and international drinking water guidelines (which predominantly employs culture-based analysis), the large-volume batch solar reactor prototypes used in the current study may effectively treat rainwater to within drinking water standards and provide water to the inhabitants of rural areas and urban informal settlements in sub-Saharan Africa."
Lines 592 to 600: "The discrepancy in the results obtained using culture- and molecular-based analyses highlights the limitations of solely using traditional culture-based analyses to monitor water treatment systems, as an over-

estimation of treatment system efficiency may be obtained. Thus, results obtained using molecular-based assays may be more representative of the viable and intact community in the treated water source, and a more accurate indication of the health risk to the end-user may be calculated when this data set is employed in quantitative microbial risk assessment (QMRA). Current research by the WATERSPOUTT research consortium is thus aimed at applying QMRA to monitor the quality of the treated rainwater."

Reviewer Three (#3):

Comment 1: Graphical abstract - This is an interesting image but might be too large to scale well to a small section on the journal website- please simplify if possible, the main content seems to be the white box with the schematic of the solar treatment apparatus

Reviewer Five (#5):

Comment 2: Graphical abstract - Too dark, better to change the background.

Based on the reviewer recommendations, the graphical abstract has been simplified and the background illustration has been removed.

Comment 2: General - The reviewed manuscript is very comprehensive and addresses the important topic of treating small-scale harvested rainwater using solar disinfection to meet drinking water needs in Cape Town, South Africa. This topic is of interest to readers of STOTEN and the manuscript reads clearly. Importantly, the authors incorporated information about the water usage and held workshops with residents to improve their understanding of issues related to rainwater safety, and developed a water safety plan template. They should be commended for this effort. Supplemental information was very detailed and comprehensive.

Thank you for the comment.

Comment 3: Abstract - Line 29 specify which anions and cations

During the chemical analyses, six anions (i.e. sulphate, chloride, nitrite, nitrate, phosphate and fluoride) and 25 cations (i.e. aluminium, antimony, arsenic, boron, barium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, phosphorus, potassium, selenium, silicon, sodium, strontium, vanadium and zinc) were monitored. Due to the journal "Abstract" word limit, all the anions and cations cannot be specified within this section.

The abstract has however been amended as follows:

Lines 28 to 31: "…all the measured physico-chemical parameters (e.g. pH and turbidity, amongst others), anions (e.g. sulphate and chloride, amongst others) and cations (e.g. iron and lead, amongst others) were within national and international drinking water guidelines limits."

All the tested anions and cations, their respective concentrations and concentration limits stipulated by the reference drinking water guidelines [i.e. South African National Standards 241 (South African Bureau of Standards, 2005); Department of Water Affairs and Forestry (1996), Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011) and World Health Organization (WHO) (2017)] are outlined in Table A.3 of the supplementary information and referred to in Lines 178, 181, 313, 316 and 341 of the main manuscript.

Comment 4: Abstract - Line 39-40: indicate of spp. or specific species

The quantitative polymerase chain reaction (qPCR) assays used for the quantification of the target organisms in the current study, were genus specific with the exception of the qPCR assay used for *Escherichia coli* (*E. coli*). The term “spp.” has been inserted in the abstract to clarify this as follows:

Lines 39 to 41: “…analysed indicator organisms (*E. coli* and enterococci spp.) and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* spp. oocysts) in the…”

Comment 5: Introduction - Line 50: The Global Risks Report is produced by the World Economic Forum- please edit citation accordingly. Please give the scale of the rankings (1-10, where 1 is low and 10 is high?)

Thank you for the comment. The citation for the Global Risks Report has been amended in-text and in the reference list as follows:

Line 52: “…(World Economic Forum, 2019).”

Line 740 to 741: “World Economic Forum., 2019. *The Global Risks Report 2019 14th Edition*. Available: http://www3.weforum.org/docs/WEF_Global_Risks_Report_2019.pdf. [2019, February 10].”

The Global Risks Report classifies the top 10 risks based on “likelihood of occurring” and “impact”. Additionally, a scale of 1 to 5 was used by respondents to classify both the “likelihood” (1: a risk that is very unlikely to occur to 5: a risk that is very likely to occur) and “impact” (1: minimal impact, 2: minor impact, 3: moderate impact, 4: severe impact and 5: catastrophic impact) of each global risk. The risk posed by “water crises” ranked 9^{th} (out of 10) in terms of likelihood and 4^{th} (out of 10) in terms of impact. The terms “rating of 9” and “rating of 4” have thus been replaced by the terms “9^{th} overall” and “4^{th} overall” in the manuscript as follows:

Lines 50 to 52: “The Global Risks Report released for 2019 listed water crises as one of the top ten risks in terms of likelihood (9^{th} overall; very likely to occur) and impact (4^{th} overall; severe impact) (World Economic Forum, 2019).”

Comment 6: Introduction - Line 57 replace "exploited" with another word like "underutilized" since exploited has negative connotations

The term “under-exploited” has been replaced with the term “underutilised” as follows:

Line 57: “…rainwater is considered an underutilised water source in sub-Saharan Africa…”

Comment 7: Introduction - Line 64-65 these pathogens are not only fecal-associated; also originating from biofilms or indigenously present? Please indicate.

We are in agreement that not all of the listed microbial contaminants, namely *Legionella*, *Klebsiella*, *Pseudomonas* and *Cryptosporidium*, within harvested rainwater are only associated with faecal matter. As indicated in Line 62, these microbial contaminants may also originate from organic debris being washed into the rainwater harvesting tank during a rain event. However, Bauer et al. (2003) and Kaushik et al. (2012) reported on the presence of *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in freshly captured rainwater, indicating that these organisms may be indigenously present within this water source. Woo et al. (2013) and Wei et al. (2016) then reported on the detection of *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp., and

Staphylococcus spp. in bioaerosol particles, which may elucidate why these organisms may also be detected in "fresh" rainwater.
The sentence regarding the origin of the microbial contaminants has been amended as follows:
Line 61 to 65: "While the chemical pollutants have not been directly associated with the incidence of disease, organic debris, faecal matter from animals that have access to the catchment surface and bioaerosol particles, have been identified as the primary sources of microbial contaminants such as *Legionella*, *Klebsiella*, *Pseudomonas* and *Cryptosporidium* (Hamilton et al., 2019)."

- Bauer, H., Giebl, H., Hitzenberger, R., Kasper-Giebl, A., Reischl, G., Zibuschka, F., Puxbaum, H., 2003. Airborne bacteria as cloud condensation nuclei. J. Geophys. Res. 108, 4658-4665. https://doi.org/10.3390/atmos10120786.
- Kaushik, R., Balasubramanian, R., De La Cruz, A.A., 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. Appl. Environ. Microbiol. 78, 2813-2818. https://doi.org/10.1128/AEM.07695-11.
- Wei, K., Zou, Z., Zheng, Y., Li, J., Shen, F., Wu, C., Wu, Y., Hu, M., Yao, M., 2016. Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing. Sci. Total Environ. 550, 751-759. https://doi.org/10.1016/j.scitotenv.2016.01.137.
- Woo, A.C., Manreetpal, S.B., Chan, Y., Lau, M.C.Y., Leung, F.C.C., Scott, J.A., Vrijmoed, L.L.P., Zawar-Reza, P., Pointing, S.B., 2013. Temporal variation in airborne microbial populations and microbially-derived allergens in a tropical urban landscape. Atmos. Environ. 74, 291-300. https://doi.org/10.1016/j.atmosenv.2013.03.047.

Comment 8: Introduction - Line 74-75 does the PET container contain phthalates? These are endocrine disruptors, is there any concern for leaching of these materials from the plastic?
As PET was not used in the construction of the large-volume batch solar reactor prototypes, the potential leaching of plasticisers from PET was not discussed in the current article. However, members of the WATERSPOUTT research consortium are currently assessing the potential leaching of endocrine disruptors from poly(methyl methacrylate) (PMMA) (also known as plexiglass), which was used in the current study for the construction of the large-volume batch solar reactor prototypes. The potential leaching of endocrine disruptors from PMMA has been included in the "Results" section as follows:
Lines 548 to 550: "The potential degradation (leaching) of the PMMA reactor tubing is however, being investigated by members of the WATERSPOUTT research consortium."

Comment 9: Introduction - Line 89, 98, 106 "unpublished results" does not appear in the reference list or supplemental documents, please cite this in reference list as unpublished manuscript, personal communication, or include description in SI
The in-text references to "unpublished results", namely Martínez-García et al. (Unpublished results A) and Martínez-García et al. (Unpublished results B) are included in the reference list as follows:

Lines 676 to 678: "Martínez-García, A., Domingos, M., Canela, M.C., Oller, I., Vincent, M., Fernández-Ibáñez, P., Polo-López, M.I., (Unpublished results A). Comparative assessment of CPC and V-trough solar reactors for the disinfection of rainwater."

Lines 679 to 681: "Martínez García, A., Polo-López, M.I., Oller, I., Vincent, M., Fernández-Ibáñez, P., (Unpublished results B). Novel large-scale solar reactor for disinfection of rainwater: assessment of a consortium of bacteria and phages."

Comment 10: Introduction - Line 112, 116: Is Salmonella considered as a frank or opportunistic pathogen? Cite reference justifying consideration as opportunistic pathogen if categorizing as such

While almost all strains of *Salmonella* are considered pathogenic (due to their ability to invade, replicate and survive in human hosts), generally, children (< 5 years old), the elderly and immunocompromised patients are more susceptible to *Salmonella* infection in comparison to healthy individuals (Eng et al. 2015). Research has also indicated that certain strains lack the ability to persist in the host cell (which is crucial for pathogenesis) and are thus non-virulent (Bakowski et al. 2008). Moreover, certain serotypes are host-specific and can only reside in one or a few animal species [e.g. *Salmonella enterica* serotype Dublin (cattle) and *Salmonella enterica* serotype Choleraesuis (swine)] (WHO, 2018). Due to the potential of the culture-based and molecular-based assays to detect a wide range of species in the *Salmonella* genus (pathogenic and opportunistic pathogenic spp.; human vs non-human specific), the term "opportunistic pathogens" was used in the current manuscript when referring to the target organisms (e.g. *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp.).

References justifying the classification of the respective target organisms as opportunistic pathogens have been added as follows:

Lines 119 to 121: "…and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.) (Fields et al., 2002; Eng et al., 2015; Clements et al., 2019; Strauss et al., 2019), while propidium…"

All added references were already available in the reference list, with the exception of Eng et al. (2015), which has subsequently been added (Lines 639 to 641).

- Bakowski, M.A., Braun, V., Brumell, J.H., 2008. *Salmonella* containing vacuoles: directing traffic and nesting to grow. Traffic. 9, 2022-2031. https://doi.org/10.1111/j.1600-0854.2008.00827.x.
- Eng, S-K., Pusparajah, P., Ab Mutalib, N-S., Ser, H-L., Chan, K-G., Lee, L-H., 2015. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Front. Life Sci. 8, 284-293. https://doi.org/10.1080/21553769.2015.1051243.
- World Health Organization (WHO)., 2018. *Salmonella* (non-typhoidal). Available: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal). [2020, January 20].

Comment 11: Introduction - Line 138 did the first flush diverter (Superhead rainwater filter) also include a filtration unit and if so what kind/pore size? Or just a diversion of first flush volume or a mesh screen?

Reviewer Five (#5):

Comment 8: Which type of first flush diversion system was installed? Smiler one in the attachment?

A Superhead® rainwater filter was installed at the site. The system contains a traditional first-flush diverter with a mesh leaf screen opening. As water flows into the unit, it is automatically diverted through the one-way filter into the flush pipe. As soon as the flush pipe is full, the clean water is diverted into the rainwater tank through an insect screen (stopping any insects or floating debris from getting into the water tank). The information has been amended as follows:

Line 142 to 143: "…, a first-flush (FF) diverter with built-in leaf and insect screens (Superhead® rainwater filter) was installed to redirect the initial roof run-off during a rain event (Fig. 1.B)."

Comment 12: Methods - Line 169-170 give a brief description of the chemical analysis process/ instrument type(s) used and which cations and anions were monitored for. Why were only a subset of samples monitored for anions and turbidity?

Representative samples were analysed for anions and turbidity as previous research conducted by members of our research group indicated that anion concentrations in rainwater collected from the region (Stellenbosch), adhered to drinking water standards (Dobrowsky et al., 2015; Reyneke et al., 2016; 2018; Strauss et al., 2016; 2018). Similarly, the rainwater samples were also found to have low levels of turbidity [<1.00 Nephelometric Turbidity Units (NTU)], which adhered to drinking water standards (Strauss et al. 2016; 2018).

A brief description of the chemical analysis process and an explanation of why representative samples were analysed for anion and turbidity concentrations has been added as follows:

Lines 175 to 183: "Briefly, for cation analysis, 50 mL Falcon™ high-clarity polypropylene tubes (Corning Life Sciences, USA) and polyethylene caps were pre-treated with 1% nitric acid before sample collection. Following sample collection, the concentration of 25 cations (outlined in Table A.3 of the supplementary information) were determined after acidification (1% ultrapure nitric acid) using inductively coupled plasma mass spectrometry (Agilent 7700 ICP-MS) by the Central Analytical Facility (CAF) at Stellenbosch University. One litre water samples were collected for anion and turbidity analyses (outlined in Table A.3 of the supplementary information) and processed by Bemlab Laboratories (Cape Town, South Africa) using a Thermo Scientific Gallery™ Automated Photometric Analyser."

Lines 185 to 191: "Representative samples were analysed for anions and turbidity as previous research conducted by members of our research group indicated that anion concentrations in rainwater collected from the region (Stellenbosch), adhered to drinking water standards (Dobrowsky et al., 2015; Reyneke et al., 2016; 2018; Strauss et al., 2016; 2018). Similarly, the rainwater samples were also found to have low levels of turbidity [<1.00 Nephelometric Turbidity Units (NTU)] which adhered to drinking water standards (Strauss et al. 2016; 2018)."

- Dobrowsky, P.H., Carstens, M., De Villiers, J., Cloete, T.E., Khan, W., 2015. Efficiency of a closed-coupled solar pasteurization system in treating roof harvested rainwater. Sci. Total Environ. 536, 206-214. https://doi.org/10.1016/j.scitotenv.2015.06.126.
- Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W., 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing *Legionella* contamination of roof-harvested rainwater. Sci. Total Environ. 553, 662-670. https://doi.org/10.1016/j.scitotenv.2016.02.108.
- Reyneke, B., Cloete, T.E., Khan, S., Khan, W., 2018. Rainwater harvesting solar pasteurization treatment systems for the provision of an alternative water source in peri-urban informal settlements. Environ. Sci: Water Res. Technol. 4, 291-302. https://doi.org/10.1039/c7ew00392g.
- Strauss A., Dobrowsky P.H., Ndlovu T., Reyneke B., Khan W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. BMC Microbiol. 16, 289. https://doi.org/10.1186/s12866-016-0909-y.
- Strauss A., Reyneke B., Waso M., Khan W., 2018. Compound parabolic collector solar disinfection system for the treatment of harvested rainwater. Environ. Sci.: Water Res. Technol. 4, 976-991. https://doi.org/10.1039/C8EW00152A.

Comment 13: Methods - Line 176 briefly describe filtration- what effective volume was analyzed for the culture samples?
Comment 14: Methods - Line 185-186 give media and conditions for Klebsiella, Pseudomonas, Salmonella spp.
Detailed information regarding the culture-based analyses for the respective indicator organisms [*E. coli*, total coliforms, faecal coliforms, enterococci and heterotrophic bacteria (HPC)] and opportunistic pathogens (*Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp.) has been added as follows:
Filtration for *E. coli* and total coliforms - Lines 196 to 201: "Briefly, a total volume of 100 mL (undiluted, 10^{-1} and 10^{-2}) was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (70 kPa). The filters were then placed onto Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) and were incubated at 35 ± 2 °C for 18 - 24 hrs."

Enterococci, faecal coliforms and HPC – Lines 201 to 209: "In order to enumerate enterococci, 100 µL of an undiluted sample was spread plated onto Slanetz and Bartley Agar (Oxoid), with the plates incubated for 44 – 48 hrs at 36 ± 2 °C (Strauss et al., 2016). In order to enumerate faecal coliforms (FC), 100 µL of an undiluted sample was spread plated onto m-FC Agar (Biolab, Merck, Wadeville, South Africa), with the plates incubated for 44 – 48 hrs at 35 ± 2 °C (Strauss et al., 2016). For the enumeration of the heterotrophic plate count/bacteria (HPC), a serial dilution (10^{-1}–10^{-3}) was prepared for each sample and by use of the spread plate method 100 µL of an undiluted sample and each dilution (10^{-1}–10^{-3}) was plated onto Luria Bertani (LB) agar (Biolab), with the plates incubated at 37 °C for up to four days."

Klebsiella spp., *Pseudomonas* spp. and *Salmonella* spp. – Lines 214 to 219: "Additionally, *Klebsiella* spp. (HiCrome™ *Klebsiella* Selective Agar; Sigma-Aldrich, St Louis, MO), *Pseudomonas* spp. (*Pseudomonas* Isolation

Agar; Sigma-Aldrich) and *Salmonella* spp. (*Salmonella-Shigella* Agar; Oxoid) were enumerated as outlined in Clements et al. (2019) by spread plating 100 µL of an undiluted sample onto the respective media and incubating the plates at 37 °C for 18 to 24 hours."

Comment 15: Methods - Line 200 indicate Cryptosporidium species (or spp.) analyzed. Why only quantify in a subset? Also indicate whether spp. or a particular species in SI table A.1

The primer set for the detection and quantification of *Cryptosporidium* oocysts targeted the general *Cryptosporidium* oocyst wall protein. *Cryptosporidium* spp. oocysts would thus be detected and quantified. The term "*Cryptosporidium* oocysts" has been replaced by "*Cryptosporidium* spp. oocysts" throughout the manuscript (Lines 41, 122, 233, 236, 453, 458, 481 and 591) and supplementary information [Table A1, Figure A8 (G)].

Unfortunately as tank water concentration methods were optimised for the EMA analysis, an insufficient volume of water was available for sampling #1 to #8 for the additional tank water concentration and PMA treatment required for *Cryptosporidium* spp. oocyst detection and quantification. The following information has been added to the manuscript:

Lines 234 to 236: "…(an insufficient volume of water was available for #1 to #8 for the additional tank water concentration and PMA treatment required for *Cryptosporidium* spp. oocyst detection and quantification)."

Comment 16: Methods - Line 246 paired t-test has underlying assumption of normality of the differences in the variables- please verify this was checked with Wilcoxon rank sum or another test and/or that the data met the assumptions of the parametric t-test.

Thank you for the comment. The information has been amended as follows:

Lines 277 to 285: "Statistical analyses were conducted utilising either RStudio (version 1.0.153) or Minitab19. Shapiro-Wilk tests were performed in order to determine whether the data was evenly or non-evenly distributed. Overall differences in sample composition between site 1 and site 2 and the untreated (Tank 1 and Tank 2) and solar reactor treated (Prototype I and II) tank water samples was then determined by evaluating all measured physico-chemical, chemical and microbial parameters using either the parametric paired t-test or the non-parametric Wilcoxon test (significant when $p < 0.05$). Principle component analysis (PCA) was then used to visualise the correlations between the measured cations at both sites and identify which cations primarily influenced the sample composition at each site."

Comment 17: Methods - Appendix 2 part 2 Hazards and hazardous events identification- also animals themselves could get caught in tanks, also for storage tank microbial contamination from buildup of biofilms, scale, algal growth, etc.

The following information has been amended in the Hazards and hazardous events identification section:

Rainwater Storage Tank:

"Microbial and physical [organic matter/plant debris, insects, small animals (rodents, lizards etc.)] contamination enters the storage tank due to a missing or inadequate (e.g. damaged, cracked, leaking, no vermin/insect cover) overflow pipe."

"Microbial and chemical contamination due to the build-up of biofilms or formation of a sediment layer in the bottom of the tank."

Comment 18: Results & Discussion - Line 418-419 average reduction in opportunistic pathogens- which pathogens did this include? Is Crypto included in this number?

The reported 74.43% reduction includes all the monitored organisms using EMA-qPCR and PMA-qPCR analysis. The sentence has been amended and the organism names (in brackets) have been added as follows:

Lines 456 to 458: "For the monitored indicator organisms and opportunistic pathogens, EMA-qPCR (*E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.) and PMA-qPCR (*Cryptosporidium* spp. oocysts) analysis…"

Comment 19: Results & Discussion - Line 423-425 this also indicates reason for caution that water meeting the guidelines is safe as this is a weakness of the FIB-regulatory paradigm

We are in agreement and the following information has been added to the manuscript to highlight the limitations of assessing water quality using only indicator bacteria:

Lines 474 to 480: "Thus while the use of indicator bacteria (culture-based analysis) has become routine when monitoring water quality, it should be noted that there is a poor correlation between the presence of faecal indicators and potential pathogenic bacteria (Ahmed et al., 2008). Monitoring for the removal of potentially pathogenic microorganisms which may have entered a VBNC state following water treatment is thus essential as these VBNC bacteria still pose a health risk as they are potentially infectious (Mansi et al., 2014)."

- Ahmed, W., Huygens, F., Goonetilleke, A., Gardner, T., 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. Appl. Environ. Microbiol. 74, 5490-5496. https://doi.org/10.1128/AEM.00331-08.
- Mansi, A., Amori, I., Marchesi, I., Marcelloni, A.M., Proietto, A.R., Ferranti, G., Magini, V., Valeriani, F., Borella, P., 2014. Legionella spp. survival after different disinfection procedures: Comparison between conventional culture, qPCR and EMA-qPCR. Microchem. J. 112, 65-69. https://doi.org/10.1016/j.microc.2013.09.017.

Comment 20: Results & Discussion - Line 428 how much regrowth was there after 24 h? It would be useful to have measurements of the molecular markers for regrowth as well to see if this trend is consistent by method. Looking at typical household water needs/ usage rates in this area, would the entire volume treated be used within 24 h?

The volume of water that was stored to monitor microbial regrowth was insufficient to conduct molecular-based analysis on the sample. However, it was hypothesised in the current study that the discrepancies observed between the culture-based and molecular-based analyses may be attributed to the presence of VBNC. These VBNC cells may then regain their ability to be cultured under favourable conditions or once the cells have initiated DNA repair mechanisms. The mean regrowth (CFU/100 mL) observed in the samples has been included in the manuscript as follows:

Lines 432 to 435: "The treated water collected from the large-volume batch solar reactor prototypes could however, only be stored for a maximum of 24 hours, as microbial regrowth occurred after this point (2.0×10^3 CFU/100 mL to 1.80×10^4 CFU/100 mL detected after 24 hours)."

Based on the minimum essential water requirement for health and hygiene of 20 L per person per day (WHO, 2013), a typical household (4 people) could use the entire volume of treated water produced by the Prototype II solar reactor (88 L), while a household of 7 people could use the volume of water produced by the Prototype I solar reactor (140 L). However, the systems that were installed aimed to serve as an alternative water source to multiple households within the community and thus the entire volume of treated water would be used on a daily basis.

- World Health Organization (WHO)., 2013. How much water is needed in emergencies. Technical notes on drinking-water, sanitation and hygiene in emergencies. Available: https://www.who.int/water_sanitation_health/publications/2011/WHO_TN_09_How_much_water_is_needed.pdf?ua=1. [2020, January 20].

Comment 21: Results & Discussion - Line 488-500 any information gathered on the efficacy of the community education program? It would be interesting to gather some information on this in the future.
Follow-up workshops are currently being conducted with participating community members from both sites, which are aimed at the development of educational material through the implementation of co-design principles and are also aimed at assessing issues based on water governance, gender-based roles related to water and water security, amongst others. As this research is ongoing and part of the larger collaborative Social Science work package of the WATERSPOUTT project, this information was not included in the current manuscript which focused on assessing the microbial and chemical quality of the water produced by the large-volume batch solar reactor prototypes.

Comment 22: Results & Discussion - Is there a "standardized" template or list of required components for a Water Safety plan from WHO or any other organization? Or did the authors develop this completely?
A recommended template for a Water Safety Plan (WSP) and required components is outlined by the WHO (2004) and was summarised in Lines 257 to 275. These main components include: (1) A simplified description of the technology, (2) Hazards and hazardous events identification and risk assessment, (3) Tools for operational monitoring and (4) Management programmes. Using this information, the WSP was compiled (Appendix B and Appendix C). However, as indicated in Appendix B, the risk assessment matrix (3X3 semi-quantitative matrix) was obtained from WHO (2009), while the checklist (outlined in the "*Tools for Operational Monitoring*" section) was obtained from the WHO (2018).

- World Health Organization (WHO)., 2004. Guidelines for drinking-water quality. Rev. 3rd ed. World Health Organization. Geneva, Switzerland: WHO Press. ISBN: 92-4-154638-7.
- World Health Organization (WHO)., 2009. Water Safety Plans. Managing drinking-water quality from catchment to consumer. By Bartram, J., Corrales, L., Davison, A., Deere, D., Drury, D., Gordon, B., Howard,

G., Rinehold, A., Stevens, M. WHO, Geneva, ISBN 978 92 4 156263 8. Available: https://apps.who.int/iris/bitstream/handle/10665/75141/9789241562638_eng.pdf?sequence=1. [2020, January 20].

- World Health Organization (WHO)., 2018. Draft Management Advice Sheet. Rainwater collection and storage. Available: https://www.who.int/water_sanitation_health/sanitation-waste/sanitation/revision-of-who-sanitary-inspection-forms/en/ [2020, January 20].

Comment 23: Results & Discussion - Based on the description in the appendix, there are many animals in the area of the solar reactors and opportunities for generation of dust, additional debris entering the SODIS reactor. Did the authors notice any substantial differences between any previous bench-scale work and the current measurements taken?

As outlined in Lines 402 to 424, results from the current study indicated that the preliminary pilot-scale analyses of the large-volume batch solar reactor prototypes (Martínez García et al., Unpublished results A) may have overestimated the treatment efficiency of the systems as compared to the results obtained in the current study, where environmental field trial samples were analysed. It was hypothesised that the presence of more resilient environmental strains (of the target organisms) may have contributed to this observation.

- Martínez García, A., Polo-López, M.I., Oller, I., Vincent, M., Fernández-Ibáñez, P., (Unpublished results B). Novel large-scale solar reactor for disinfection of rainwater: assessment of a consortium of bacteria and phages.

Comment 24: Results & Discussion - Could metal ions from the roofing material interfere with the disinfection process? Are metals something that should be measured to make sure they aren't increased during heat treatment?

The concentration of metals should definitely be monitored when assessing water quality and water treatment systems, specifically during heat treatment, as long-term exposure to increased metal concentrations may pose a health risk to the end-user (Martin and Griswold, 2009). While investigating the efficiency of solar pasteurization systems to treat roof-harvested rainwater, members of our research group reported on the leaching of metals (e.g. iron, aluminium and copper amongst others) from the stainless steel storage tank of a solar pasteurization system (Dobrowsky et al., 2015; Reyneke et al., 2016). As the measured concentrations exceeded the limits stipulated by various drinking water guidelines, it was recommended that the storage tank of the solar pasteurization systems be constructed from a high-density polyethylene. Subsequent studies (Strauss et al., 2016; Reyneke et al., 2018) using a new solar pasteurization system (with a high-density polyethylene storage tank) indicated that decreased leaching of metals occurred inside the new systems; however, as metal pipe connectors were still used in the new solar pasteurization system, leaching of copper and nickel occurred.

Based on these results, the use of metal components in the design of the large-volume batch solar reactor prototypes in the current study was limited to the system frame, with no metal components exposed to the rainwater during treatment. This was confirmed as no significant difference in cation concentration was observed between the untreated and treated rainwater samples at each site (Table A3). The observed cation concentrations recorded in the untreated and treated rainwater samples can thus be attributed to metals (e.g. Zinc) leaching from the metal roofing material during the harvesting process. It has been reported that the presence of metal oxides (eg. ZnO and Fe_2O_3) in the water can contribute to the generation of reactive oxygen species and thus increase SODIS treatment efficiency (Byrne et al., 2011). As the measured cation concentrations were within the drinking water guidelines, the potential leaching of metal components during water treatment was not discussed.

- Byrne, J.A., Fernandez-Ibañez, P., Dunlop, P.S.M., Alrousan, D.M.A., Hamilton, J.W.J., 2011. Photocatalytic enhancement for solar disinfection of water: a review. Int. J. Photoenergy. 2011, 798051. https://doi.org/10.1155/2011/798051.
- Dobrowsky, P.H., Carstens, M., De Villiers, J., Cloete, T.E., Khan, W., 2015. Efficiency of a closed-coupled solar pasteurization system in treating roof harvested rainwater. Sci. Total Environ. 536, 206-214. https://doi.org/10.1016/j.scitotenv.2015.06.126.
- Martin, S., Griswold, W., 2009. Human health effects of heavy metals. Environmental Science and Technology Briefs for Citizens. 15, 1-6.
- Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W., 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing Legionella contamination of roof-harvested rainwater. Sci. Total Environ. 553, 662-670. https://doi.org/10.1016/j.scitotenv.2016.02.108.
- Reyneke, B., Cloete, T.E., Khan, S., Khan, W., 2018. Rainwater harvesting solar pasteurization treatment systems for the provision of an alternative water source in peri-urban informal settlements. Environ. Sci: Water Res. Technol. 4, 291-302. https://doi.org/10.1039/c7ew00392g.
- Strauss A., Dobrowsky P.H., Ndlovu T., Reyneke B., Khan W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. BMC Microbiol. 16, 289. https://doi.org/10.1186/s12866-016-0909-y.

Comment 25: Results & Discussion - A pathogen that was not considered- Naegleria- Fig A.4 you could also discourage nasal rinsing.

Thank you for the comment. We are in agreement that *Naegleria* may be a protozoan pathogen of concern in harvested rainwater and members of our research group have investigated the presence of specifically *Naegleria fowleri* in harvested rainwater (Waso et al. 2008) and the inactivation of *Naegleria fowleri* using a solar pasteurization system (Dobrowsky et al. 2016). In the current study, *Cryptosporidium* spp. oocysts were monitored as a representative of protozoan contaminants as *Cryptosporidium* has been recommended as a protozoan water quality indicator (WHO, 2016) and Heaselgrave and Kilvington (2011) reported that *Cryptosporidium* oocysts (0.14 to 0.32 log reduction) were more UV resistant as compared to *Acanthamoeba* cysts (2.16 to 3.84 log reduction), *Naegleria* cysts (3.59 to 3.84 log reduction), *Entamoeba* cysts (1.90 to 1.92 log reduction), *Giardia* cysts (1.94 to 1.96 log reduction) and *Ascaris* ova (0.56 to 1.42 log reduction).

The risk posed by nasal rinsing is definitely an exposure scenario we will take into consideration in future research as we will be conducting quantitative microbial risk assessment to elucidate the risk associated with using untreated and treated rainwater. The activities outlined on the information poster were selected based on the domestic activities commonly performed within the target communities and were identified based on community surveys [Water Research Commission (WRC) Project Report K5/2368//3, 2016] and personal communication with the target communities.

- Dobrowsky, P.H., Khan, S., Cloete, T.E., Khan, W., 2016. Molecular detection of *Acanthamoeba* spp., *Naegleria fowleri* and *Vermamoeba* (*Hartmannella*) *vermiformis* as vectors for *Legionella* spp. in untreated and solar pasteurized harvested rainwater. Parasite. Vector. 9, 539. https://doi.org/10.1186/s13071-016-1829-2.
- Heaselgrave, W., Kilvington, S., 2011. The efficacy of simulated solar disinfection (SODIS) against *Ascaris*, *Giardia*, *Acanthamoeba*, *Naegleria*, *Entamoeba* and *Cryptosporidium*. Acta Tropica. 119, 138-143. https://doi.org/10.1016/j.actatropica.2011.05.004.
- Waso, M., Dobrowsky, P.H., Hamilton, K.A., Puzon, G., Miller, H., Khan, W., Ahmed, W., 2018. Abundance of *Naegleria fowleri* in roof-harvested rainwater tank samples from two continents. Environ. Sci. Pollut. Res. Int. 25, 5700-5710. https://doi.org/10.1007/s11356-017-0870-9.
- Water Research Commission (WRC), Project No. K5/2368//3., 2016. *Design, Construction and Monitoring of Sustainable Domestic Rainwater Harvesting Treatment Systems in Enkanini Informal Settlement, Stellenbosch*. Report to the Water Research Commission, Project No. K5/2368 by Department of Microbiology, Stellenbosch University. Stellenbosch, South Africa.
- World Health Organization (WHO)., 2016. Results of round 1 of the WHO international scheme to evaluate household water treatment technologies. World Health Organization. https://apps.who.int/iris/handle/10665/204284.

Reviewer Five (#5):

Comment 1: Even though it is not very systematic, the results of this field study is important to report. Frequent and extreme weather events make traditional methods inefficient to provide safe drinking water to rural communities and alternative methods, such as the method suggested in this study may be useful. The paper in mainly discusses the microbial water quality and it is better to reflect it in the title, abstract and introduction. Following comments are given to further improve the manuscript.

Thank you for the comment. A comprehensive analysis of the chemical quality of the untreated and treated rainwater was also conducted in the current study (six physico-chemical parameters, 6 anions and 25 cations monitored). These results are summarised in Section 3.1 ("*Physico-chemical properties and chemical analysis of the collected tank water samples*"), while the measured concentrations and comparison to the respective drinking water quality guidelines are outlined in Table A3.

Comment 3: Better to add a figure with technical specifications. For example, pipe diameter, thickness, lengths, heights, bed angle, orientation, RW tank elevation etc.

Information regarding the component dimensions and the installation of the tanks is summarised in-text (Lines 127 to 143) and outlined in detail in Appendix A of the Supplementary Information ("*Description of sampling*

sites”). However, a schematic diagram (Fig. A3) outlining the measurements of the large-volume batch solar reactor prototypes (and components) has been added to Appendix A of the Supplementary Information. The numbering of the supplementary figures has been updated in Appendix A and main manuscript accordingly.
Reference to the information in the Supplementary Information has been amended in the manuscript as follows:
Lines 143 to 146: “A detailed description of the sampling sites, system installation and schematic diagrams of the large-volume batch solar reactors is outlined in Appendix A, while additional information regarding the working mechanism of the large-volume batch solar reactors is outlined in Appendix B.”

Comment 4: Better to show treatment performance with expose to sunlight (no of hours and light intensity).
Information regarding treatment time, the mean UV-A and UB-B irradiance (W/m^2) recorded during each solar reactor treatment and temperature (°C) of the collected samples (untreated, treated and total increase in temperature) is outlined in Table A2 (Appendix A, Supplementary Information). Table A2 is referenced in Lines 296, 300 and 304 of the main manuscript.

Comment 5: Better to use rainfall data to justify the concept.
The daily rainfall and ambient temperatures recorded for each day during the 2018/2019 sampling period is outlined in Fig. A6 (Appendix A, Supplementary Information). To clarify this the manuscript has been amended as follows:
Lines 289 to 293: “The daily rainfall and ambient temperatures recorded throughout the 2018/2019 research period as well as the sampling sessions for each site are depicted in Fig. A.6. A total rainfall of 431.4 mm was recorded during July 2018 to September 2018 (high rainfall period), while 183.8 mm was recorded during October 2018 to January 2019 (medium rainfall period). The rainfall then decreased to 146.2 mm during February to April 2019 (low rainfall period).”

Comment 6: Introduction - research question has not been well defined, also use resent publications in similar studies (ex. SENEVIRATHNA, S., RAMZAN, S. & MORGAN, J. 2019. A sustainable and fully automated process to treat stored rainwater to meet drinking water quality guidelines. Process Safety and Environmental Protection, 130, 190-196.).
The current study aimed to assess water treatment systems that could cost-effectively be implemented in developing countries (such as rural areas and urban informal settlements). As such, the introduction aimed to highlight “solar disinfection” (SODIS) as a treatment technology that is currently used within developing countries (Lines 70 to 81) and outline the various limitations that have been identified with using this technique (Lines 81 to 84). These limitations, namely treatment volume and treatment efficiency may then be overcome through the use of SODIS enhancement technologies (Lines 84 to 87). Although we applied the SODIS enhancement technologies to design the large-volume batch solar reactor prototypes and assess their efficiency in controlled pilot-scale studies (Lines 88 to 102), it was necessary to assess the efficiency of the systems on-site in the communities for which these systems had been designed (aim of the current study – Lines 108 to 124). In order to clarify this, the following information has been added to the introduction:
Lines 103 to 107: “Although the preliminary pilot-scale assessment of the solar reactor prototypes display promise in treating rainwater, it is crucial that these systems be assessed on-site in the target communities, i.e.

rural areas and urban informal settlements. This will allow for a more comprehensive indication as to whether these reactors may serve as a sustainable solution in providing communities with a safe alternative water source."

The recommended Senevirathna et al. (2019) reference has been included in-text and in the reference list as follows:
Lines 66 to 70: "Treatment strategies that may be implemented to improve the quality of rainwater include the utilisation of gutter screens or first-flush diverters for the prevention of contaminant entry into the collection tank or post-collection treatment [chemical (e.g. chlorination) and physical treatments (e.g. filtration, solar disinfection (SODIS) and thermal disinfection)] (Hamilton et al., 2019; Senevirathna et al., 2019)."

Lines 713 to 715: "Senevirathna, S.T.M.L.D., Ramzan, S., Morgan, J., 2019. A sustainable and fully automated process to treat stored rainwater to meet drinking water quality guidelines. Process Saf. Environ. 130, 190-196. https://doi.org/10.1016/j.psep.2019.08.005."

Comment 7: Peoples acceptance is important in this type of projects. You better discuss community centred design principles applied in the design and provide evidence to for people's acceptance of this idea.
We are in agreement that the target community members need to be taken into consideration when designing and implementing water treatment systems. Due to the success with which simple SODIS has been implemented in developing countries, the European Union funded WATERSPOUTT (Water Sustainable Point Of Use Treatment Technologies) project (grant agreement no. 688928) aimed to investigate cost-effective and efficient solar-based treatment technologies, with socio-economic sciences and humanities also included in the project (Net4Society, 2018). The development of the solar-based treatment technologies by the WATERSPOUTT research consortium took 2 years and involved dialogue and co-design with the end-users in the target communities as well as the completion of social surveys. The initial prototype of the large-volume batch solar reactors included an aeration system and heating panel to increase treatment efficiency. However, based on the results obtained from these pilot-scale studies and engagement with the community members (assessing their water needs, material availability and economic means) the current prototypes were developed. The results from these shared dialogue workshops could not be included in the current manuscript as the main focus of the current study was to assess the treatment efficiency of the systems on-site in the target communities (focussing on basic sciences), with the results obtained by the socio-economic sciences and humanities partners being prepared for an independent publication. However, an example of the co-design principles followed by our WATERSPOUTT research partners in Malawi for the development of solar-ceramic filtration devices is already publicly available (Buck et al. 2017; Morse et al. 2018).

- Buck, L., Morse, T., Lungu, K., Petney, M., 2017. Interactional co-design and co-production through shared dialogue workshops. In: 2017 International Conference on Engineering and Product Design Education. 7 to 8 September 2017. Oslo and Akerhus University College of Applied Sciences, Norway. Available: https://bucks.repository.guildhe.ac.uk/17350/1/17350_Buck_L.pdf. [2020, January 20].

- Morse, T., Lungu, K., Luwe, K., Chiwalua, L., Mulwafu, W., Buck, L., Harlow, R., Honor, F., McGuigan, K., 2018. A transdisciplinary co-design and behaviour change approach to introducing SODIS to rural communities in Malawi. In: 2018 Water and Health Conference: Where Science Meets Policy, 29 October to 2 November 2018. University of North Carolina, United States of America. Available: https://strathprints.strath.ac.uk/66270/1/Morse_UNCWHC_2018_A_transdisciplinary_co_design_and_behaviour_change_approach.pdf. [2020, January 20].
- Net4Society., 2018. WATERSPOUTT: a success story in SSH integration. Available: https://www.net4society.eu/files/Net4Society_D_3_3_FINAL_Factsheets_SSH_Integration.pdf. [2020, January 20].

Comment 9: Sampling protocol is very important in this study, which is not well explained in this paper.
In order to clarify the sample collection procedure, the section has been amended as follows:
Lines 152 to 159: "For the microbial and chemical analysis of the water produced by the solar reactor prototypes (Fig. 1), an untreated 10 L sample was collected directly from the RWH tank at each site [hereafter referred to as Tank 1 (Site 1) and Tank 2-FF (Site 2)] on the morning of a sampling event. The respective solar reactor prototypes at each site were then immediately filled with tank water from the RWH tanks and exposed to direct sunlight for 6 hours (sampling sessions 1 to 8) or 8 hours (sampling sessions 9 to 18). Following the completion of the solar exposure, 10 L of each solar treated sample was collected directly from the solar reactors [hereafter referred to as Prototype I (Site 1) and Prototype II (Site 2)]."

Comment 10: Figure 3 - this results seems both tank and prototypes, better to change the figure title.
Thank you for the comment. The figure legend has been amended as follows:
"**Fig. 3.** Box and whiskers plot illustrating the distribution of the intact cells or oocysts/100 mL recorded for each of the target organisms using EMA-qPCR (*E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp.) and PMA-qPCR (*Cryptosporidium* spp. oocysts) in the untreated (T1 and T2-FF; solid blue box) and treated (PI and PII; dashed red box) tank water samples collected from (A) site 1 and (B) site 2. The whiskers at the end of each box indicate the minimum and maximum values, while the box is defined by the lower and upper quartiles and the mean value."

Comment 11: The overall procedure seems not systematic, first run the systems with 6 hrs, then increased to 8 hours. If the authors can provide the variation of treatment efficiency with time, it will be more useful to determine optimum time of exposure.
Based on the results obtained by Martínez-García et al. (Unpublished results A) and Martínez-García et al. (Unpublished results B), a 6 hour solar exposure treatment time was identified as sufficient to reduce microbial contaminants in synthetic rainwater. The field-trials were thus initially conducted using a 6 hour solar exposure time. However, based on the results that were obtained [heterotrophic bacteria (HPC) detected in the treated water at levels exceeding the drinking water standards] the treatment time was increased to 8 hours in order to see whether the increased treatment time would allow for the reduction of HPC to within drinking water standards.

The variation in treatment efficiency based on treatment time is visually represented in Fig. A.8 (Appendix A, Supplementary Information). Additionally, as the total UV exposure will determine treatment efficiency, the mean UV-A and UV-B irradiance (W/m^2) recorded during each solar reactor treatment (sampling session 1 to 18) is outlined in Table A2 (Appendix A, Supplementary Information). As outlined in Lines 416 to 422, the increase in treatment time from 6 hours to 8 hours resulted in the mean UV radiation increasing from 20.82 $W/m^2/h$ (6 hour treatment) to 24.72 $W/m^2/h$ (8 hour treatment). Correspondingly, results indicated that the mean HPC log removal increased from ≥ 1.21 log (6 hour treatment) (Line 411) to ≥ 2.02 log (8 hour treatment) (Line 424).

- Martínez-García, A., Domingos, M., Canela, M.C., Oller, I., Vincent, M., Fernández-Ibáñez, P., Polo-López, M.I., (Unpublished results A). Comparative assessment of CPC and V-trough solar reactors for the disinfection of rainwater.
- Martínez García, A., Polo-López, M.I., Oller, I., Vincent, M., Fernández-Ibáñez, P., (Unpublished results B). Novel large-scale solar reactor for disinfection of rainwater: assessment of a consortium of bacteria and phages.

Comment 12: Line 396-397 do you have results? Otherwise provide the references.
The statement, "The robustness of system components therefore also needs to be taken into consideration when designing water treatment systems for use in rural areas and informal settlements, where replacement components may not be readily available.", was made based on our research groups' experience with assessing water treatment systems on-site in informal settlements (Reyneke et al., 2018). We have noted that the operational sustainability and maintenance of system components may determine whether a treatment technology can successfully be integrated into a target community. Similar observations were made by Mwabi et al. (2011) and McGuigan et al. (2012). The references have been included in-text (Line 553) and were added to the reference list.

- McGuigan, K.G., Conroy, R.M., Mosler, H., Du Preez, M., Ubomba-Jaswa, E., Fernandez-Ibañez, P., 2012. Solar water disinfection (SODIS): a review from bench-top to roof-top. J. Hazard. Mater. 235-236, 29-46. https://doi.org/10.1016/j.jhazmat.2012.07.053.
- Mwabi, J.K., Adeyemo, F.E., Mahlangu, T.O., Mamba, B.B., Brouckaert, B.M., Swartz, C.D., Offringa, G., Mpenyana-Monyatsi, L., Momba, M.N.B., 2011. Household water treatment systems: A solution to the production of safe drinking water by the low-income communities of Southern Africa. Phys. Chem. Earth. 36, 1120-1128. https://doi.org/10.1016/j.pce.2011.07.078.

Comment 13: Operational and maintenance issues encountered are worth to report. Particularly the effect of pipe aging on treatment efficiency, algae growth in the pipes, pipe sedimentation issues, and replacement of parts in prototypes.
As outlined in Lines 545 to 548, monitoring of the operational sustainability of the solar reactor prototypes at both sites indicated that system maintenance was limited to cleaning the surface of the PMMA reactor tubes (prevent dust accumulation that will influence UV transmittance), with no system components needing replacement during the study period.

Additionally, the installation of the first-flush diverter system at site 2 reduced the entry of organic matter into the rainwater harvesting tank. While at both sites 1 and 2 the outlet tap of the rainwater harvesting tank was located approximately 10 cm from the bottom of the tank. It would therefore be possible for sedimentation to occur inside the rainwater harvesting tank; however, a build-up of organic matter or sedimentation did not occur inside the large-volume batch solar reactor prototypes. Poly(methyl methacrylate) (PMMA) was selected for use in the construction of the solar reactor prototypes as this plastic is considered durable and less likely to scratch. A reduction in the treatment efficiency of the large-volume batch solar reactor prototypes was not observed over the 9-month monitoring period during the current study.

Comment 14: It is worth to indicate the cost of a prototypes ($/prototype), operational cost ($/month) and production cost ($/L) and compare these numbers with other reported rainwater treatment systems.
Based on the current large-volume batch solar reactor designs (88 to 140 L treatment volumes) it is estimated that the production cost of treated water will range from US$ 0.01/L to US$ 0.14/L. This estimate was made based on the assumption that the solar reactor prototypes are used eight months of the year (243 days) to treat their full capacity (88 L and 140 L, respectively) and that the systems would have a life expectancy of 8 years. It is however important to note that the current cost estimate may be decreased by replacing the high-grade aluminium framework with a more cost-effective alternative, while large-scale production of the systems will also decrease construction cost.

Information regarding the cost analyses and a summary table (Table A5) comparing different household water treatment technologies has been inserted in Appendix A as follows:
Main manuscript Lines 553 to 555: "A preliminary cost analysis for the solar reactor prototypes has been included in Appendix A, with the cost (US$/L) compared to the costs associated with other household drinking water treatment systems (Table A.5)."

Supplementary Information, Appendix A:
Table A5 Estimated cost analysis of the solar reactor prototypes and comparison to other used household water treatment systems.

Treatment System	Cost (US$/L)	Reference
Large-volume batch solar reactors	0.0 - 0.14	Current Study
Traditional SODIS (2 L)	0.0016	Keogh et al. (2015)
SODIS using a 19L Water Dispenser Container	0.0021	Keogh et al. (2015)
25L SODIS compound parabolic collector	0.002	Ubomba-Jaswa et al. (2010)
Chlorination	0.0007 - 0.1	Sobsey et al. (2008); Shrestha et al. (2018)
Ceramic filtration	0.0018	Shrestha et al. (2018)
Boiling using gas	0.011	Shrestha et al. (2018)
Boiling electricity	0.017	Shrestha et al. (2018)
Reverse osmosis and UV treatment	0.026	Shrestha et al. (2018)

- Keogh, M.B., Castro-Alférez, M., Polo-López, M.I., Fernández Calderero, I., Al-Eryani, Y.A., Joseph-Titus, C., Sawant, B., Dhodapkar, R., Mathur, C., McGuigan, K.G., Fernández-Ibáñez, P., 2015. Capability of 19-L polycarbonate plastic water cooler containers for efficient solar water disinfection (SODIS): Field case studies in India, Bahrain and Spain. Sol. Energy. 116, 1-11. https://doi.org/10.1016/j.solener.2015.03.035.
- Shrestha, K.B., Thapa, B.R., Aihara, Y., Shrestha, S., Bhattarai, A.P., Bista, N., Kazama, F., Shindo, J., 2018. Hidden cost of drinking water treatment and its relation with socioeconomic status in Nepalese urban context. Water. 10, 607. https://doi.org/10.3390/w10050607.
- Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I., McGuigan, K., 2010. Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. J. Chem. Tech. Biotech. 85, 1028-1037. https://doi.org/10.1002/jctb.2398.

Thank you for your time and co-operation.

Yours sincerely

Wesaal Khan
Associate Professor (Microbiology)

Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa

B. Reyneke[a], T. Ndlovu[a], M.B. Vincent[b], A. Martínez-García[c], M.I. Polo-López[c], P. Fernández-Ibáñez[c,d], G. Ferrero[e], S. Khan[f], K.G. McGuigan[g] and W. Khan[a*]

[a] Department of Microbiology, Faculty of Science, Stellenbosch University, Private Bag X1, Stellenbosch, 7602, South Africa.

[b] Ecosystem Environmental Services S.A., Sant Andreu de Llavaneres, Barcelona, Spain.

[c] Plataforma Solar de Almeria-CIEMAT, P.O. Box 22, Tabernas, Almería, Spain.

[d] Nanotechnology and Integrated BioEngineering Centre, School of Engineering, University of Ulster, Newtownabbey, Northern Ireland, United Kingdom.

[e] IHE Delft Institute for Water Education, Westvest 7, 2611, AX, Delft, the Netherlands.

[f] Faculty of Health Sciences, University of Johannesburg, PO Box 17011, Doornfontein, 2028, South Africa.

[g] Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland.

Short title: Large-volume batch SODIS treatment of rainwater

Abbreviations[1]

*Corresponding Author: Wesaal Khan; Phone: +27218085804; Fax: +27218085846; Email: wesaal@sun.ac.za

[1] ADWG – Australian drinking water guidelines; BDL – below detection limit; CFU – colony forming units; CPC – compound parabolic collector; DNA – deoxyribonucleic acid; DWAF – Department of Water Affairs and Forestry; *E. coli* – *Escherichia coli*; EMA – ethidium monoazide bromide; EU – European Union; FF – first-flush; HPC – heterotrophic plate count/heterotrophic bacteria; LB – luria bertani; PCA – principle component analysis; PET – polyethylene-terephthalate; PMA – propidium monoazide; PMMA – poly(methyl methacrylate); qPCR – quantitative polymerase chain reaction; RHRW – roof-harvested rainwater; ROS – reactive oxygen species; RWH – rainwater harvesting; SABS – South African Bureau of Standards; SODIS – solar disinfection; UV – ultraviolet radiation; WATERSPOUTT – Water Sustainable Point-Of-Use Treatment Technologies; WHO – World Health Organisation; WSP – water safety plan; Zn – zinc.

Abstract

The efficiency of two large-volume batch solar reactors [Prototype I (140 L) and II (88 L)] in treating rainwater on-site in a local informal settlement and farming community was assessed. Untreated [Tank 1 and Tank 2-(First-flush)] and treated (Prototype I and II) tank water samples were routinely collected from each site and all the measured physico-chemical parameters (e.g. pH and turbidity, amongst others), anions (e.g. sulphate and chloride, amongst others) and cations (e.g. iron and lead, amongst others) were within national and international drinking water guidelines limits. Culture-based analysis indicated that *Escherichia coli*, total and faecal coliforms, enterococci and heterotrophic bacteria counts exceeded drinking water guideline limits in 61%, 100%, 45%, 24% and 100% of the untreated tank water samples collected from both sites. However, an 8 hour solar exposure treatment for both solar reactors was sufficient to reduce these indicator organisms to within national and international drinking water standards, with the exception of the heterotrophic bacteria which exceeded the drinking water standard limit in 43% of the samples treated with the Prototype I reactor (1 log reduction). Molecular viability analysis subsequently indicated that mean overall reductions of 75% and 74% were obtained for the analysed indicator organisms (*E. coli* and enterococci spp.) and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* spp. oocysts) in the Prototype I and II solar reactors, respectively. The large-volume batch solar reactor prototypes could thus effectively provide four (88 L Prototype II) to seven (144 L Prototype I) people on a daily basis with the basic water requirement for human activities (20 L). Additionally, a generic Water Safety Plan was developed to aid practitioners in identifying risks and implement remedial actions in this type of installation in order to ensure the safety of the treated water.

Keywords: Rainwater harvesting; Large-volume SODIS reactors; EMA-qPCR; rainwater quality; water scarcity

1. Introduction

The Global Risks Report released for 2019 listed water crises as one of the top ten risks in terms of likelihood (9th overall; very likely to occur) and impact (4th overall; severe impact) (World Economic Forum, 2019). The probability of a water crisis risk in sub-Saharan Africa is significantly increased as a high proportion of the population reside in urban informal settlements (densely populated areas with inadequate water and municipal services) and rural areas, with limited access to a safe water supply and waste disposal and sanitation infrastructure (Dos Santos et al., 2017). However, as highlighted by Gwenzi and Nyamadzawo (2014) and Emenike et al. (2017), rainwater is considered an underutilised water source in sub-Saharan Africa and may serve as an effective reserve to improve and encourage equity in water access. Roof-harvested rainwater (RHRW) can however, be contaminated with various chemicals and microorganisms, which may limit its use as a potable water source (Hamilton et al., 2019). While the chemical pollutants have not been directly associated with the incidence of disease, organic debris, faecal matter from animals that have access to the catchment surface and bioaerosol particles, have been identified as the primary sources of microbial contaminants such as *Legionella*, *Klebsiella*, *Pseudomonas* and *Cryptosporidium* (Hamilton et al., 2019).

Treatment strategies that may be implemented to improve the quality of rainwater include the utilisation of gutter screens or first-flush diverters for the prevention of contaminant entry into the collection tank or post-collection treatment [chemical (e.g. chlorination) and physical treatments (e.g. filtration, solar disinfection (SODIS) and thermal disinfection)] (Hamilton et al., 2019; Senevirathna et al., 2019). Although various chemical and physical treatment technologies have been investigated, SODIS is considered a cost-effective treatment method and is recommended by the World Health Organisation (WHO) for the effective reduction of microbial contamination in water sources (Ubomba-Jaswa et al., 2010). In its simplest form, SODIS entails filling a transparent container [usually a 2 L polyethylene-terephthalate (PET) bottle] with contaminated water and exposing the bottle to direct sunlight for six to eight hours to allow ultraviolet (UV) radiation and solar-mild heat to inactivate microbial contaminants (McGuigan et al., 2012). Ultraviolet radiation directly inactivates the microbial contaminants by damaging nucleic acids and leads to the formation of reactive oxygen species (ROS), which react and damage proteins, nucleic acids and membrane lipids (Nelson et al., 2018). The water temperature will also increase as water molecules absorb the UV radiation, which leads to cell membrane damage (≥ 45 °C) (McGuigan et al., 2012). The major drawbacks associated with this technique are the small volumes of water that can effectively be treated (2 to 5 L) and decreased efficiency during overcast weather conditions (requiring up to 48 hours of treatment). Increases in treatment volume and efficiency may then be obtained by employing various modifications (SODIS enhancement technologies) such as solar mirrors (concentrates UV radiation) and larger reactor tubes (increase treatment volume) (Ubomba-Jaswa et al., 2010; McGuigan et al., 2012).

As part of the European Union (EU) Horizon 2020 WATERSPOUTT project (grant agreement no. 688928), Martínez-García et al. (Unpublished results A) investigated various enhancement technologies that may cost-effectively allow for larger volumes of water to be treated using SODIS. Results from the study indicated that the use of a static batch reactor system employing V-trough solar mirrors allowed for the effective treatment of a larger volume (68% more) of water compared to the compound parabolic collector (CPC)-type solar mirrors under the same solar exposure conditions. In a follow-up study, the same research group designed two large-volume batch solar reactor prototypes (static batch systems with 88 L and 140 L treatment volumes, respectively), where multiple poly(methyl methacrylate) (PMMA) reactor tubes were positioned in the centre of V-trough solar mirrors (Martínez-García et al., Unpublished results B). Preliminary assessment of the solar reactor prototypes, using spiked synthetic rainwater samples and culture-based analysis, indicated that a ≥ 6 log removal efficiency was obtained for *Escherichia coli* (*E. coli*) and *Salmonella enteriditis* after 1.5 hour natural sunlight exposure, while a 2 hour sunlight exposure was required to achieve the same log reduction for *Enterococcus faecalis* and *Pseudomonas* *aeruginosa* (*P. aeruginosa*). Although the preliminary assessment of the solar reactor prototypes display promise in treating rainwater, it is crucial that these systems be assessed on-site in the target communities, i.e. rural areas and urban informal settlements. This will allow for a more comprehensive indication as to whether these reactors may serve as a sustainable solution in providing communities with a safe alternative water source.

The primary aim of the current study was thus to assess the efficiency of the two newly designed WATERSPOUTT large-volume batch solar reactor prototypes (Martínez-García et al., Unpublished results B) for the treatment of RHRW on-site in a local informal settlement (140 L Prototype I) and a rural farming community (88 L Prototype II). The chemical quality of the RHRW before and after solar reactor treatment was routinely assessed by monitoring various physico-chemical parameters (e.g. temperature, pH, and turbidity), anions and cations. Additionally, the removal of traditional indicator organisms (*E. coli*, total and faecal coliforms, enterococci and heterotrophic bacteria) and selected opportunistic pathogens (*Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp.), was assessed using culture-based analysis. Ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) assays were also used to monitor the reduction efficiency of indicator organisms (*E. coli* and enterococci) and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.) (Fields et al., 2002; Eng et al., 2015; Clements et al., 2019; Strauss et al., 2019), while propidium monoazide (PMA) qPCR assays were used to monitor *Cryptosporidium* spp. oocyst reductions. A Water Safety Plan (WSP) outlining guidelines for the use of rainwater harvesting combined with solar reactor treatment was also implemented, as this may aid in ensuring the safety of the treated RHRW.

2. Materials and methods

2.1 Description of the large-volume batch solar reactor prototypes and sampling sites

Two large-volume batch solar reactor prototypes were designed and constructed as part of the WATERSPOUTT project (grant agreement no. 688928) for implementation in South Africa and Uganda, with the current study focusing on the application of these systems in field trials in South Africa. The Prototype I solar reactor (140 L treatment volume) was installed in Enkanini informal settlement (Site 1; GPS coordinates: 33°55'28.1"S 18°50'35.8"E) during July 2018 and consisted of three PMMA reactor tubes (200 mm diameter) that were positioned in the centre of a V-trough solar mirror (constructed from anodized aluminium). The reactor tubes were positioned at a 34° angle (equal to the local latitude to optimise the average annual solar UV irradiance input to the solar reactor) and were inter-connected by UV-A transparent PMMA tubing (Fig. 1.A). The Prototype II solar reactor (88 L treatment volume) was installed next to a local church building in the Skoolplaas farming community (Site 2; GPS coordinates: 33°56'38.5"S 18°46'26.3"E) during July 2018 and consisted of the same materials and design as Prototype I, with the exception that eight PMMA tubes (100 mm diameter) were substituted for the three 200 mm diameter tubes used in the Prototype I system (Fig. 1.B). Additionally, as space was available between the gutter system and the rainwater harvesting (RWH) tank at site 2, a first-flush (FF) diverter with built-in leaf and insect screens (Superhead® rainwater filter) was installed to redirect the initial roof run-off during a rain event (Fig. 1.B). A detailed description of the sampling sites, system installation and schematic diagrams of the large-volume batch solar reactors is outlined in Appendix A, while additional information regarding the working mechanism of the large-volume batch solar reactors is outlined in Appendix B.

2.2 Ethical clearance and sample collection

Exemption from ethical clearance was obtained from the Research Ethics Committee (Humanities) Stellenbosch University (Ethics Reference no.: SU-HSD-004624), as the participating households were instructed to only use the treated water for domestic uses and not for drinking purposes.

For the microbial and chemical analysis of the water produced by the solar reactor prototypes (Fig. 1), an untreated 10 L sample was collected directly from the RWH tank at each site [hereafter referred to as Tank 1 (Site 1) and Tank 2-FF (Site 2)] on the morning of a sampling event. The respective solar reactor prototypes at each site were then immediately filled with tank water from the RWH tanks and exposed to direct sunlight for 6 hours (sampling sessions 1 to 8) or 8 hours (sampling sessions 9 to 18). Following the completion of the solar exposure, 10 L of each treated sample was collected directly from the solar reactors [hereafter referred to as Prototype I (Site 1) and Prototype II (Site 2)]. Based on the availability of rainwater in the RWH tanks, 15 sampling sessions were conducted at site 1 (n = 30; August 2018 to March 2019), while 18 sampling sessions were conducted at site 2 (n = 36; August 2018 to April 2019). For ease of presentation, sampling sessions 1 to 18 are designated as #1 (sampling session 1), #2 (sampling session 2), etc., throughout the manuscript.

The temperature, pH, electrical conductivity and total dissolved solids present in all water samples were measured using a hand-held Milwaukee Instruments MI806 meter (Spraytech, South Africa), while the dissolved oxygen was measured using a Milwaukee Instruments M600 meter (Spraytech, South Africa). Rainfall and daily ambient temperature data for the study period was obtained from the South African Weather Services, while solar irradiance data [mean ambient UV-A and UV-B radiation] was obtained from the Stellenbosch Weather Services [Stellenbosch University, Faculty of Engineering (http://weather.sun.ac.za/)].

2.3 Chemical analysis

The chemical quality of the untreated and solar reactor treated tank water samples was determined by monitoring cation and anion concentrations and measuring sample turbidity (Strauss et al. 2018). Briefly, for cation analysis, 50 mL Falcon™ high-clarity polypropylene tubes (Corning Life Sciences, USA) and polyethylene caps were pre-treated with 1% nitric acid before sample collection. Following sample collection, the concentration of 25 cations (outlined in Table A.3 of the supplementary information) were determined after acidification (1% ultrapure nitric acid) using inductively coupled plasma mass spectrometry (Agilent 7700 ICP-MS) by the Central Analytical Facility (CAF) at Stellenbosch University. One litre water samples were collected for anion and turbidity analyses (outlined in Table A.3 of the supplementary information) and processed by Bemlab Laboratories (Cape Town, South Africa) using a Thermo Scientific Gallery™ Automated Photometric Analyser. All samples (*n* = 66) were monitored for cations, while representative samples (*n* = 22; #1, #7, #10, #12, #15 and #18) were monitored for anions and turbidity. Representative samples were analysed for anions and turbidity as previous research conducted by members of our research group indicated that anion concentrations in rainwater collected from the region (Stellenbosch), adhered to drinking water standards (Dobrowsky et al., 2015; Reyneke et al., 2016; 2018; Strauss et al., 2016; 2018). Similarly, the rainwater samples were also found to have low levels of turbidity [<1.00 Nephelometric Turbidity Units (NTU)] which adhered to drinking water standards (Strauss et al. 2016; 2018).

2.4 Culturing of indicator organisms and opportunistic pathogens

The microbial quality of the tank water samples collected from sites 1 and 2 was monitored before (untreated) and after solar reactor treatment using various culture-based analyses. *Escherichia coli* and total coliforms were enumerated simultaneously using membrane filtration as described by Dobrowsky et al. (2015). Briefly, a total volume of 100 mL (undiluted, 10^{-1} and 10^{-2}) was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (70 kPa). The filters were then placed onto Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) and were incubated at 35 ± 2 °C for 18 - 24 hrs. In order to enumerate enterococci, 100 µL of an undiluted sample was spread plated onto Slanetz and Bartley

Agar (Oxoid), with the plates incubated for 44 – 48 hrs at 36 ± 2 °C (Strauss et al., 2016). In order to enumerate faecal coliforms (FC), 100 µL of an undiluted sample was spread plated onto m-FC Agar (Biolab, Merck, Wadeville, South Africa), with the plates incubated for 44 – 48 hrs at 35 ± 2 °C (Strauss et al., 2016). For the enumeration of the heterotrophic plate count/bacteria (HPC), a serial dilution (10^{-1}–10^{-3}) was prepared for each sample and by use of the spread plate method 100 µL of an undiluted sample and each dilution (10^{-1}–10^{-3}) was plated onto Luria Bertani (LB) agar (Biolab), with the plates incubated at 37 °C for up to four days. For the treated samples (Prototypes I and II) where the HPC were reduced to below the detection limit [BDL; < 1 colony forming units (CFU)/1 mL], the potential regrowth of bacteria was monitored. Briefly, 20 mL of each treated sample was stored in a sterile McCartney bottle at room temperature and 100 µL of the treated water was spread plated onto LB agar (Biolab, Merck) every 24 hours for a period of 2 days. The plates were then incubated at 37 °C. Additionally, *Klebsiella* spp. (HiCrome™ *Klebsiella* Selective Agar; Sigma-Aldrich, St Louis, MO), *Pseudomonas* spp. (*Pseudomonas* Isolation Agar; Sigma-Aldrich) and *Salmonella* spp. (*Salmonella-Shigella* Agar; Oxoid) were enumerated as outlined in Clements et al. (2019) by spread plating 100 µL of an undiluted sample onto the respective media and incubating the plates at 37 °C for 18 to 24 hours. Additionally, coliphages were enumerated as outlined by Baker et al. (2003) using *E. coli* ATCC 13706 as the target bacterial host. All culture-based analyses were performed in duplicate.

2.5 Tank water concentration, viability treatment and DNA extraction

The concentration of 1 L (Site 1) and 2 L (Site 2) samples, EMA treatment and subsequent DNA extractions were performed for each of the samples collected before and after solar reactor treatment as outlined in Reyneke et al. (2016). An increased sample volume was processed for site 2 in order to obtain sufficient DNA for the subsequent molecular-based analysis. For the molecular quantification of *Cryptosporidium* spp. within the collected samples, the same methodology was repeated with the exception that a PMA treatment as described by Alonso et al. (2014) was followed.

2.6 Molecular-based enumeration of indicator organisms and opportunistic pathogens

Quantitative PCR was performed in order to quantify *E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp. in all of the collected tank water samples, while *Cryptosporidium* spp. oocysts were quantified in the samples collected from #9 to #15 and #9 to #18 for sites 1 and 2, respectively (an insufficient volume of water was available for #1 to #8 for the additional tank water concentration and PMA treatment required for *Cryptosporidium* spp. oocyst detection and quantification). All qPCR assays were conducted using a LightCycler® 96 (Roche Diagnostics, Risch-Rotkreuz, Switzerland) instrument in combination with the FastStart Essential DNA Green Master Mix (Roche Diagnostics) as outlined in Reyneke et al. (2017), with the primer pairs and cycling parameters presented in Table A.1. Standard curves for the respective qPCR assays were generated using the methodology outlined in Reyneke et al. (2017), while the qPCR performance characteristics of the various assays were analysed using the Roche LightCycler® 96 Software Version 1.1. Furthermore, to compensate for the different sample volumes used per site for rainwater concentration [1 L (Site 1) and 2 L (Site 2)] the gene copies detected in the samples utilising the qPCR assays were converted to gene copies per 100 mL of the original tank water sample as outlined by Waso et al. (2018). The gene copy numbers (gene copies/100 mL) were then converted to cell equivalents (cells or oocysts/100 mL) by utilising the number of copies of the target gene present within the target host (Table A.1). All final concentrations for qPCR analyses are thus presented as equivalent cells or oocysts/100 mL original tank water sample.

2.7 Maintenance of prototype reactors and water safety plan

Following the system installations, workshops were conducted within the respective communities to outline the principle of rainwater harvesting, the working mechanism and operational maintenance of the solar reactors (Fig. A.4). Information on the domestic activities (i.e. laundry, cleaning, washing, etc.) the treated rainwater could be used for was also provided (Fig. A.5).

As outlined by the WHO (2004), the most efficient way of consistently ensuring the safety of a drinking water supply is through the utilisation of a WSP (Appendix B), which is defined as a risk assessment and management approach that monitors the entire water supply process (e.g. collection of RHRW to utilisation of treated water by the consumer). The first step in the development of the WSP was to develop a simplified guide to RWH and the use of the solar reactor prototypes that would provide the end-users with a basic description of the technology and guidelines for the implementation and maintenance of the system (Appendix B). This was achieved by identifying all potential hazards and hazardous events that may influence the quality of rainwater during the harvesting, storage and treatment process (Appendix B), using published literature and personal observations at the respective study sites, during the study period. Thereafter, various maintenance and remedial actions were identified to prevent certain water safety hazards (e.g. prevent organic debris from entering the storage tank) or to implement after a hazardous event occurred (e.g. control measure failed and organic debris washed into the storage tank) (Appendix B). Following the identification of the potential hazards, a risk assessment matrix (Appendix C) was compiled that would enable the risk characterisation associated with each hazard/hazardous event and enable the assessment of the various control measures (e.g. maintenance strategies, use of a first-flush diverter system etc.) in eliminating or minimising the identified water safety hazards.

2.8 Statistical analysis

Statistical analyses were conducted utilising either RStudio (version 1.0.153) or Minitab19. Shapiro-Wilk tests were performed in order to determine whether the data was evenly or non-evenly distributed. Overall differences in sample composition between site 1 and site 2 and the untreated (Tank 1 and Tank 2) and solar reactor treated (Prototype I and II) tank water samples was then determined by evaluating all measured physico-chemical, chemical and microbial parameters using either the parametric paired *t*-test or the non-parametric Wilcoxon test (significant when $p < 0.05$). Principle component analysis (PCA) was then used to visualise the correlations between the measured cations at both sites and identify which cations primarily influenced the sample composition at each site.

3. Results and Discussion

3.1 Physico-chemical properties and chemical analysis of the collected tank water samples

The daily rainfall and ambient temperatures recorded throughout the 2018/2019 research period as well as the sampling sessions for each site are depicted in Fig. A.6. A total rainfall of 431.4 mm was recorded during July 2018 to September 2018 (high rainfall period), while 183.8 mm was recorded during October 2018 to January 2019 (medium rainfall period). The rainfall then decreased to 146.2 mm during February to April 2019 (low rainfall period). The mean ambient UV-A radiation at both sampling sites ranged from 7.16 W/m^2 (12/09/2018) to 31.29 W/m^2 (14/01/2019), while the mean ambient UV-B radiation ranged from 1.33 W/m^2 (12/09/2018) to 4.63 W/m^2 (14/01/2019) (Table A.2). The untreated tank water temperature at site 1 (Tank 1) ranged from 9.0 °C (02/08/2018 and 15/08/2018) to 24.0 °C (28/01/2019), with a mean temperature of 16.3 °C recorded for all sampling days, while the tank water temperature in the samples collected from the Prototype I solar reactor ranged from 15.5 °C (12/09/2018) to 45.0 °C (28/01/2019) (mean 28.9 °C) (Table A.2). Similarly, the untreated tank water temperature at site 2 (Tank 2-FF) ranged from 10.0 °C (15/08/2018) to 26.0 °C (25/10/2018) (mean 18.1 °C), while the tank water temperature in the samples collected from the Prototype II solar reactor ranged from 18.0 °C (12/09/2018) to 46.5 °C (28/01/2019) (mean 32.6 °C) (Table A.2).

All measured physico-chemical parameters (pH, turbidity, electrical conductivity, total dissolved solids and dissolved oxygen) in the collected untreated and prototype treated rainwater samples adhered to the drinking water guideline limits of the South African

Department of Water Affairs and Forestry (DWAF) (DWAF, 1996), South African National Standards (SANS) 241 [South African Bureau of Standards (SABS), 2005], Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and WHO (2017), with no significant difference ($p > 0.05$) observed for the data collected for the untreated and treated (Tank 1 and Prototype I; Tank 2-FF and Prototype II) tank water samples or between sites 1 and 2 (Tank 1 and 2-FF) (Table A.3).

Results for the chemical analyses of the untreated (Tank 1 and Tank 2-FF) and treated (Prototype I and Prototype II) tank water samples collected from sites 1 and 2, indicated that all anions and cations (Table A.3) were within the respective drinking water guideline limits [DWAF, 1996; SANS 241 (SABS, 2005); ADWG (NHMRC and NRMMC, 2011); WHO, 2017], with the exception of the mean zinc (Zn) concentration recorded in the samples collected from site 1 [Tank 1 (mean of 3044 μg/L) and Prototype I (mean of 3061 μg/L)]; which exceeded (albeit not significantly) the DWAF (1996) and ADWG (NHMRC and NRMMC, 2011) limit of 3000 μg/L. However, these samples were within the 5000 μg/L SANS 241 (SABS, 2005) limit. The increased Zn concentrations recorded at site 1 (Tank 1 and Prototype I), in comparison to site 2 (Tank 2-FF and Prototype II), may primarily be attributed to the Zn metal sheeting roofing material used to construct the catchment system, as the leaching of metals from metal roofing materials (corrosion during rain events and continuous exposure to sunlight) have been reported to be a major contributor of metal ions in rainwater (Chang et al., 2004; Reyneke et al., 2018). It should be noted, that while the catchment system at site 2 was also constructed from Zn sheeting roofing material, the entire surface of the catchment system was painted with a weather resistant roof paint (personal communication) which may have limited the leaching of metal ions into the rainwater. Additionally, the first-flush diverter connected to the rainwater tank at site 2 (Tank 2-FF) may have improved the physico-chemical quality of the tank water samples. First-flush diverter systems act as a pre-treatment barrier by redirecting the initial roof run-off water (at the start of a rain event), which is thought to contain the highest concentration of pollutants (Sánchez et al., 2015). Gikas and Tsihrintzis (2012) compared the quality of RHRW collected in the flush pipe of first-flush diverter systems, with the RHRW entering the collection tanks (RWH tanks) and reported that all measured mean anion and cation concentrations were higher in the collected first-flush samples. The authors concluded that the diversion of the first-flush roof run-off away from the collection tanks improved the physico-chemical quality of the RHRW.

As no significant difference was obtained when comparing the anion and cation concentrations (Table A.3) recorded in the untreated tank water samples to the treated tank water samples (Tank 1 vs Prototype I, Tank 2-FF vs Prototype II) and the tank water samples from each site clustered together (Fig. 2), it was concluded that the solar reactor prototypes (system components and the treatment mechanism) did not influence the chemical quality of the tank water samples.

3.2 Removal efficiency of indicator bacteria and opportunistic pathogens

3.2.1 Culture-based analysis

For the untreated tank water samples collected from site 1 (Tank 1; $n = 15$), the *E. coli*, faecal coliform, total coliform, enterococci and HPC concentrations exceeded the respective drinking water guideline limits in 67%, 73%, 100%, 20% and 100% of the samples, respectively (Table 1). Analysis of the corresponding treated samples (Prototype I; $n = 15$) indicated that the *E. coli* (> 0.78 log reduction), enterococci (> 3.48 log reduction) and faecal coliform (> 4.08 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all the collected samples. Total coliforms were reduced to BDL in 63% of the treated samples collected following a 6 hour solar exposure (# 1-8) (> 3.94 log reduction), with a mean of 55 CFU/100 mL detected in the samples (37%) where total coliform counts above the standard were detected. An increase in solar exposure to 8 hours (# 9-15) resulted in an increased treatment efficiency, as total coliforms were reduced to within the 5 CFU/100 mL DWAF (1996) and 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits in 100% of the treated samples (4.66 log reduction). For the HPC analysis, 38% of the treated samples were reduced to within the drinking water guideline limit of 1.0×10^4 CFU/100 mL (1.71 log reduction) after a 6 hour solar exposure [mean of 2.4×10^4 CFU/100 mL detected in the remaining 63% samples (1.21 log reduction)], while 57% of the treated samples were reduced to below the guideline limit (2.08 log reduction) after an 8 hour solar exposure [mean of 2.7×10^4 CFU/100 mL detected in the remaining 43% of samples (1.01 log reduction)] (Fig. A.8).

For the untreated tank water samples collected from site 2 (Tank 2-FF; n = 18), the *E. coli*, faecal coliform, total coliform, enterococci and HPC concentrations exceeded the respective drinking water guideline limits in 56%, 22%, 100%, 28% and 100% of the samples, respectively (Table 1). Analysis of the corresponding treated samples (Prototype II; n = 18) indicated that the *E. coli* (> 0.48 log reduction), enterococci (> 3.34 log reduction) and faecal coliform (> 3.04 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all collected samples, while total coliforms were reduced to below the 5 CFU/100 mL DWAF (1996) and 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits (3.85 log reduction). Heterotrophic bacteria were then reduced to below the 1.0×10^4 CFU/100 mL DWAF (1996) drinking water guideline limit in 88% of the treated samples (mean of 4.6×10^3 CFU/100 mL recorded) after a 6 hour solar exposure (# 1-8) (2.11 log reduction), with a mean of 1.8×10^4 CFU/100 mL detected in the samples (12%) where HPC concentrations above the standard were detected. In comparison, 100% of the treated samples were reduced to below the 1.0×10^4 CFU/100 mL drinking water guideline limit after an 8 hour solar exposure (# 9-18) (≥ 2.02 log reduction; Fig. A.8).

Klebsiella spp. were detected in 100% (mean concentration of 1.9×10^4 CFU/100 mL) and *Salmonella* spp. in 60% (mean concentration of 6.3×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 1 (Tank 1); however, both organisms were reduced to BDL (> 4.28 and > 3.8 log reduction, respectively) following treatment using the Prototype I solar reactor (Table 1). *Klebsiella* spp. were also detected in 17% (mean concentration of 8.0×10^2 CFU/100 mL) and *Salmonella* spp. in 6% (mean concentration of 1.0×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 2 (Tank 2-FF), with both organisms reduced to BDL (> 2.9 and > 3 log reduction, respectively) following treatment using the Prototype II solar reactor (Table 1). *Pseudomonas* spp. and coliphages were not detected in any of the rainwater samples collected from sites 1 and 2.

Although numerous studies have investigated the use of SODIS to treat contaminated water, varying degrees of treatment efficiency (0.46 to > 6 log reductions in bacteria) have been reported depending on experimental design (McGuigan et al., 2012; Hamilton et al., 2019). However, a limitation of SODIS which has consistently been highlighted by these investigators is the small treatment volume (2 to 5 L). Ubomba-Jaswa et al. (2010) investigated the use of a 25 L SODIS reactor (methacrylate tube) situated inside a CPC and reported on the complete inactivation of *E. coli*, even during unfavourable weather conditions (cloudy with low solar intensity). Martínez-García et al. (Unpublished results B) then expanded on this research and investigated cost-effective SODIS enhancement strategies that would enable the treatment of larger volumes of water (32 L and 54 L), with the results obtained leading to the design of the large-volume batch solar reactor prototypes (Prototype I and II) assessed in the current study. The treatment efficiency of the Prototype I and II solar reactors was also assessed by Martínez-García et al. (Unpublished results B) under controlled conditions, by spiking synthetic rainwater with laboratory strains of *E. coli*, enterococci, *Salmonella* and *Pseudomonas* (10^5 – 10^6 CFU/mL bacterial cells) using a 6 hour solar exposure treatment time. A ≥ 6 log reduction of all the test bacteria was obtained, with the system classified as “highly protective (≥ 4 log reduction)” against bacteria according to the WHO (2016) household water treatment technology performance criteria. In comparison, results from the current study, for both solar reactor prototypes, during a 6 hour solar exposure treatment, indicated that a ≥ 2.54 log reduction was obtained when monitoring the removal of enterococci, faecal and total coliforms, while mean log reductions of ≥ 1.21 log were obtained for the removal of HPC. Based on these results, the 6 hour solar exposure treatment with the prototypes in field trials failed to meet the ≥ 2 log removal required for a “protective” classification against bacteria. The Martínez-García et al.

(Unpublished results B) study was however, conducted in a hot arid climate (Tabernas Desert, Almería, Southern Spain) with a mean UV radiation of 28.31 W/m^2/h recorded during the 6 hour treatment trials, while the field trials of the systems in the current study were conducted in a moderate Mediterranean climate (Stellenbosch, Western Cape, South Africa), where a mean UV radiation of 20.82 W/m^2/h was recorded during the 6 hour treatment trials (Table A.2).

The treatment time in the current study was subsequently increased to 8 hours (Site 1: #9-15; Site 2: #9-18) in order to increase the overall UV dose (mean UV radiation of 24.72 W/m^2/h was recorded from #9-18). For both prototypes a ≥ 3.44 log reduction was subsequently obtained when monitoring the removal of enterococci, faecal and total coliforms, while the mean log reductions for the removal of HPC increased to ≥ 2.02 log. Based on the observed treatment efficiencies obtained using the Prototype I and II solar reactors in the current study (8 hour treatment), the prototypes may be classified as "protective (≥ 2 log reduction)", for the removal of bacteria in the tank water (WHO, 2016). More importantly, culture-based analysis indicated that both treatment systems were able to produce water that adhered to the microbial parameters as stipulated in the respective drinking water guidelines [DWAF, 1996; SANS 241 (SABS, 2005); ADWG (NHMRC and NRMMC, 2011); WHO, 2017], with lower indicator organism counts recorded in the tank water samples collected from site 2, where the first-flush diverter system was installed. The treated water collected from the large-volume batch solar reactor prototypes could however, only be stored for a maximum of 24 hours, as microbial regrowth occurred after this point (2.0×10^3 CFU/100 mL to 1.80×10^4 CFU/100 mL detected after 24 hours).

3.2.2 Molecular-based analysis

The performance characteristics of the respective qPCR assays are provided in Table A.4. Results obtained using EMA-qPCR indicated that an overall mean decrease of 83.76% (0.79 log reduction) in intact *E. coli* cells was recorded after treatment using Prototype I, while an overall mean decrease of 82.76% (0.76 log reduction) was recorded after treatment for

Prototype II (Fig. 3). Similarly, intact enterococci cells decreased by a mean of 91.68% (1.08 log reduction) after treatment using Prototype I, while an 84.89% (0.82 log reduction) mean decrease was recorded after treatment using Prototype II (Fig. 3). In comparison, quantification of intact *Klebsiella* cells indicated that this genus was more resistant to the solar reactor treatment as mean decreases of 62.44% (0.43 log reduction) and 60.42% (0.40 log reduction) were recorded after treatment using Prototype I and II, respectively (Fig. 3). Similarly, intact *Legionella* cells decreased by 68.61% (0.50 log reduction) after treatment using Prototype I and by 63.77% (0.44 log reduction) after treatment using Prototype II (Fig. 3). Overall mean decreases in intact *Pseudomonas* cells of 79.09% (0.68 log reduction) and 87.50% (0.90 log reduction) were recorded after treatment using Prototype I and II, respectively, while *Salmonella* cells decreased by 78.36% (0.66 log reduction) after treatment using Prototype I and 67.82% (0.49 log reduction) after treatment with Prototype II (Fig. 3). Lastly, PMA-qPCR analysis indicated that *Cryptosporidium* spp. oocysts decreased by 57.14% (0.62 log reduction) after treatment using Prototype I, while a mean decrease of 73.81% (0.58 log reduction) was recorded after treatment using Prototype II (Fig. 3).

For the monitored indicator organisms and opportunistic pathogens, EMA-qPCR (*E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.) and PMA-qPCR (*Cryptosporidium* spp. oocysts) analysis indicated that a mean overall reduction of 74.43% was obtained following treatment for both the Prototype I and II solar reactors. This discrepancy in the observed treatment efficiency in comparison to the results obtained using culture-based analysis, may be attributed to EMA-qPCR and PMA-qPCR detecting viable but non culturable (VBNC) cells within the water samples (Fittipaldi et al., 2012; Mansi et al., 2014). It has been reported that certain opportunistic pathogens (e.g. *Legionella pneumophila* and *P. aeruginosa*) can enter a VBNC state in which they are not detectable using standard culture-based analysis but are still viable and retain their virulence (Mansi et al., 2014). Moreover, these VBNC microorganisms may regain their ability to be cultured under favourable conditions, which corresponds to the observed bacterial regrowth observed after 24 hours (culture-based analysis). Strauss et al. (2019) then applied Illumina next-generation sequencing coupled with EMA viability treatment to identify the primary pathogenic or opportunistic pathogenic genera, capable of surviving SODIS-CPC treatment in a 10.6 L CPC-reactor (Strauss et al., 2019). Results from the study indicated that intact and potentially viable bacterial cells belonging to 11 different bacterial genera (e.g. *Acinetobacter*, *Campylobacter*, *Legionella*, *Mycobacterium* and *Pseudomonas* amongst others) were detected in the SODIS-CPC treated tank water. Thus while the use of indicator bacteria (culture-based analysis) has become routine when monitoring water quality, it should be noted that there is a poor correlation between the presence of faecal indicators and potential pathogenic bacteria (Ahmed et al., 2008). Monitoring for the removal of potentially pathogenic microorganisms which may have entered a VBNC state following water treatment is thus essential as these VBNC bacteria still pose a health risk as they are potentially infectious (Mansi et al., 2014).

While the survival of the *Cryptosporidium* spp. oocysts after SODIS treatment using the solar reactor prototypes, may be attributed to the resilient nature of the oocyst wall (Hamilton et al., 2018), the ability of the opportunistic pathogenic bacteria (*Pseudomonas* spp., *Salmonella* spp., *Legionella* spp. and *Klebsiella* spp.) to survive large-volume solar-based disinfection strategies has been attributed to their ability to initiate various stress-response mechanisms and switch to a more tolerant phenotype upon exposure to environmental stressors, such as temperature and UV exposure (Jones, 1997; Fux et al., 2005). These stress-responses may include the production of heat shock proteins and the initiation of DNA repair mechanisms, amongst others (Fields et al., 2002; Breidenstein et al., 2011). For example, Srivastava et al. (2008) indicated that the overexpression of the sigma factor *algT*, protects *Pseudomonas* spp. from heat stress and allows these organisms to persist during unfavourable conditions, while DNA repair mechanisms may be initiated in response to UV-induced DNA damage, through the activation of the SOS-regulon (upregulation of *recA* and *lexA* genes) or the photolyase enzyme (Zenoff et al., 2006). Similarly, Bojer et al. (2010) attributed the heat resistance of *K. pneumoniae* to the *clpK* genetic marker, which has been shown to correlate positively with thermotolerant phenotypes observed among clinical *Klebsiella* isolates. Microorganisms have also been reported to produce pigments or structures that may enable their survival under unfavourable conditions, as has been reported for *P. aeruginosa*, where the production of pyocyanin has been hypothesised to protect *P. aeruginosa* from oxidative stress (inactivation mechanism of SODIS) (Hendiani et al., 2019). It is thus evident that microorganisms may employ numerous strategies to survive disinfection treatment and that additional treatment barriers may be required to reduce the survival of these target pathogens within water treatment systems. These strategies may include the addition of a cost-effective filtration system as a pre-treatment strategy to reduce microbial load entering the large-volume batch solar reactor prototypes (Hamilton et al., 2019).

3.3 Water safety plan, end-user engagement and operational sustainability of the systems

As numerous factors may influence the quality of RHRW during the harvesting and/or treatment process, a WSP (Appendix B) for the utilisation of rainwater harvesting in combination with the large-volume batch solar reactor prototypes was developed. As the WSP was developed concurrently with the monitoring of the large-volume batch solar reactor prototypes during the field trials, the effectiveness of the various control measures was assessed by comparing site 1 with site 2, as these sites were located in two distinct settings that could be influenced by different anthropogenic activities and potential pollution sources as outlined in Appendix A.

The application of the WSP to characterise the risk associated with RHRW collected at sites 1 and 2, indicated that the external hazards at site 1 (informal settlement) posed a greater risk of contamination. The increased risk was primarily attributed to the influence of potential pollution sources present near the catchment system (e.g. garbage disposal site, surface run-off), tree branches obstructing a section of the conveyance system, organic debris (e.g. dust/soil dispersed from the dirt pathway, leaves from the tree) within the conveyance system and corrosion of the metal sheeting catchment system.

Correspondingly, chemical and microbial analysis of the untreated tank water samples collected from sites 1 and 2 revealed that the untreated tank water collected from site 1 had higher levels of chemical contaminants (e.g. cations) and microbial contaminants in comparison to site 2. For example, the concentration of HPC was 0.72 log [3.50×10^5 CFU/100 mL (Tank 1) vs 6.90×10^4 CFU/100 mL (Tank 2-FF)] greater in the untreated tank water samples from site 1 (Tank 1), in comparison to site 2 (Tank 2-FF).

The improved tank water quality at site 2 may also be attributed to the efficiency of the implemented control measures at this site. The catchment surface at site 2 was painted with a weather resistant roof paint (personal communication) that may have reduced the leaching of metal contaminants into the collected tank water. Additionally, due to space availability a first-flush diverter was connected between the catchment system and Tank 2-FF, which served as a control measure to reduce the introduction of organic debris into the collection tank. However, the efficiency of a first-flush diverter is dependent on the maintenance of the system, which entailed cleaning/emptying the first-flush diverter after each rain event. The quality of RHRW collected from site 1 may then be improved by removing the obstructing tree branches (source of organic debris), implementing a regular gutter cleaning regime, installing a gutter screen at the inlet of the RWH tank (due to space limitation a first-flush diverter could not be connected to the current catchment system) and replacing the corroded metal sheeting on the catchment system or painting the catchment system with a weather resistant roof paint.

As previously indicated, workshops were conducted with participating households within the respective communities to outline the operational maintenance of the large-volume batch solar reactor prototypes and rainwater harvesting systems (Fig. A.4 and Fig. A.5). Subsequent monitoring of the operational sustainability of the solar reactor prototypes at both sites indicated that system maintenance was limited to cleaning the surface of the PMMA reactor tubes (prevent dust accumulation that will influence UV transmittance), with no system components needing replacement during the study period. The potential degradation (leaching) of the PMMA reactor tubing is however, being investigated by members of the WATERSPOUTT research consortium. The robustness and cost of system components should therefore be taken into consideration when designing water treatment systems for use in rural areas and informal settlements, where replacement components may not be readily available (Mwabi et al., 2011; McGuigan et al., 2012). A preliminary cost analysis for the solar reactor prototypes has been included in Appendix A, with the cost (US$/L) compared to the costs associated with other household drinking water treatment systems (Table A.5). During the study period, households who had access to the treated tank water (Prototype I and II) at sites 1 (13 households) and site 2 (5 households), primarily reported using the treated tank water for domestic activities such as cleaning of their homes, laundry and washing.

As noted by Mahmud et al. (2007), the aim of a WSP for small community water supplies should be to achieve an overall and sustained reduction in microbial contaminants/sanitary risks, rather than aim for the complete removal of microbial contaminants. The WSP outlined in the current study thus serves to reduce the contamination of RHRW by reducing "preventable contaminant entry" (e.g. organic debris and faecal matter containing an increased microbial load from washing into the storage tank) into the storage tank, whereafter treatment with the large-volume batch solar reactor prototypes may further reduce the microbial contaminants to within drinking water standards.

4. Conclusions

The physico-chemical and chemical quality of the Tank 1 and 2-FF and Prototype I and II treated rainwater samples adhered to the respective drinking water guidelines, with an improvement in quality observed at site 2 where the first-flush diverter was installed. Lower indicator bacterial counts were also recorded in the tank water samples collected from site 2 (Tank 2-FF and Prototype II) where the first-flush diverter was installed and fewer hazards were identified that may influence the tank water quality (WSP), in comparison to site 1 (Tank 1 and Prototype I). The installation of a first-flush diverter system may thus serve as an inexpensive pre-treatment strategy that may improve the overall quality of RHRW, while the establishment of a WSP may aid in identifying potential hazards and hazardous events that may influence water safety.

Both solar reactors were able to significantly reduce the level of microbial contamination in the tank water samples for all microbial indicators evaluated, to below the drinking water guideline limits [with the exception of HPC in the Prototype I treated samples (43%)], through the use of an 8 hour solar radiation exposure. Although HPC exceeding the DWAF (1996) drinking water guideline limit were recorded in 43% of the Prototype I treated samples, a mean 1.01 log reduction in heterotrophic bacteria was recorded for these samples, which would decrease the health risk associated with using the treated rainwater (in comparison to the utilisation of untreated rainwater). Based on national and international drinking water guidelines (which predominantly employs culture-based analysis), the large-volume batch solar reactor prototypes used in the current study may effectively treat rainwater to within drinking water standards and provide water to the inhabitants of rural areas and urban informal settlements in sub-Saharan Africa. Results from the EMA-qPCR and PMA-qPCR analysis however, indicated that *E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* spp. oocysts were reduced by 74.43% in both reactor prototypes. The discrepancy in the results obtained using culture- and molecular-based analyses highlights the limitations of solely using traditional culture-based analyses to monitor water treatment systems, as an over-estimation of treatment system efficiency may be obtained. Thus, results obtained using molecular-based assays may be more representative of the viable and intact community in the treated water source, and a more accurate indication of the health risk to the end-user may be calculated when this data set is employed in quantitative microbial risk assessment (QMRA). Current research by the WATERSPOUTT research consortium is thus aimed at applying QMRA to monitor the quality of the treated rainwater.

Conflicts of interests

The authors have no conflicts to declare.

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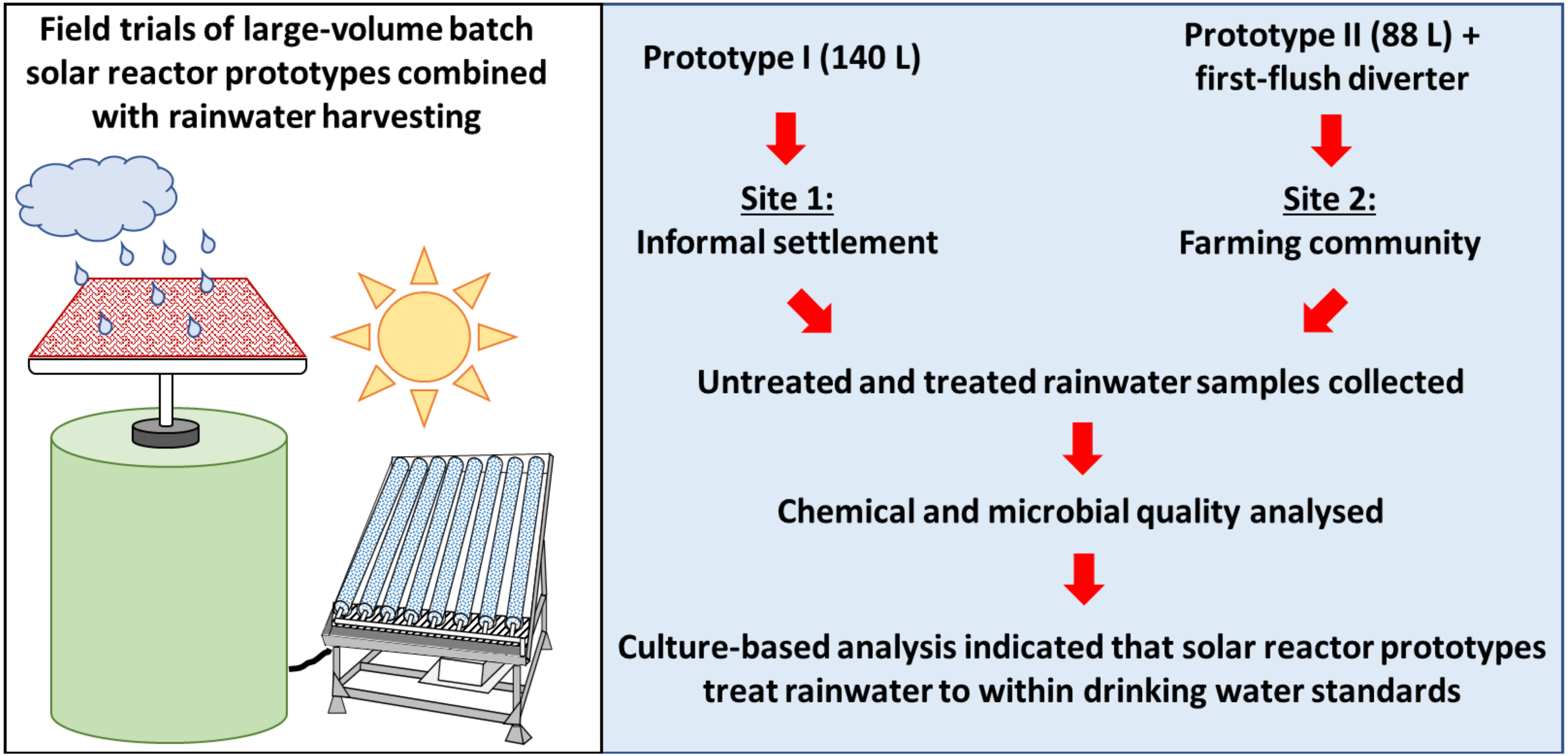
*Graphical Abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- 88 L and 140 L solar reactors treat rainwater to within drinking water standards
- EMA- and PMA-qPCR indicate a mean reduction of 74% in opportunistic pathogens
- First-flush diverter able to improve chemical and microbial quality of rainwater
- Water safety plan for rainwater harvesting and large-scale solar reactors developed

Validation of large-volume batch solar reactors for the treatment of rainwater in field trials in sub-Saharan Africa

B. Reyneke[a], T. Ndlovu[a], M.B. Vincent[b], A. Martínez-García[c], M.I. Polo-López[c], P. Fernández-Ibáñez[c,d], G. Ferrero[e], S. Khan[f], K.G. McGuigan[g] and W. Khan[a*]

[a] Department of Microbiology, Faculty of Science, Stellenbosch University, Private Bag X1, Stellenbosch, 7602, South Africa.

[b] Ecosystem Environmental Services S.A., Sant Andreu de Llavaneres, Barcelona, Spain.

[c] Plataforma Solar de Almeria-CIEMAT, P.O. Box 22, Tabernas, Almería, Spain.

[d] Nanotechnology and Integrated BioEngineering Centre, School of Engineering, University of Ulster, Newtownabbey, Northern Ireland, United Kingdom.

[e] IHE Delft Institute for Water Education, Westvest 7, 2611, AX, Delft, the Netherlands.

[f] Faculty of Health Sciences, University of Johannesburg, PO Box 17011, Doornfontein, 2028, South Africa.

[g] Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland.

Short title: Large-volume batch SODIS treatment of rainwater

Abbreviations[1]

*Corresponding Author: Wesaal Khan; Phone: +27218085804; Fax: +27218085846; Email: wesaal@sun.ac.za

[1] ADWG – Australian drinking water guidelines; BDL – below detection limit; CFU – colony forming units; CPC – compound parabolic collector; DNA – deoxyribonucleic acid; DWAF – Department of Water Affairs and Forestry; *E. coli* – *Escherichia coli*; EMA – ethidium monoazide bromide; EU – European Union; FF – first-flush; HPC – heterotrophic plate count/heterotrophic bacteria; LB – luria bertani; PCA – principle component analysis; PET – polyethylene-terephthalate; PMA – propidium monoazide; PMMA – poly(methyl methacrylate); qPCR – quantitative polymerase chain reaction; RHRW – roof-harvested rainwater; ROS – reactive oxygen species; RWH – rainwater harvesting; SABS – South African Bureau of Standards; SODIS – solar disinfection; UV – ultraviolet radiation; WATERSPOUTT – Water Sustainable Point-Of-Use Treatment Technologies; WHO – World Health Organisation; WSP – water safety plan; Zn – zinc.

Abstract

The efficiency of two large-volume batch solar reactors [Prototype I (140 L) and II (88 L)] in treating rainwater on-site in a local informal settlement and farming community was assessed. Untreated [Tank 1 and Tank 2-(First-flush)] and treated (Prototype I and II) tank water samples were routinely collected from each site and all the measured physico-chemical parameters (e.g. pH and turbidity, amongst others), anions (e.g. sulphate and chloride, amongst others) and cations (e.g. iron and lead, amongst others) were within national and international drinking water guidelines limits. Culture-based analysis indicated that *Escherichia coli*, total and faecal coliforms, enterococci and heterotrophic bacteria counts exceeded drinking water guideline limits in 61%, 100%, 45%, 24% and 100% of the untreated tank water samples collected from both sites. However, an 8 hour solar exposure treatment for both solar reactors was sufficient to reduce these indicator organisms to within national and international drinking water standards, with the exception of the heterotrophic bacteria which exceeded the drinking water standard limit in 43% of the samples treated with the Prototype I reactor (1 log reduction). Molecular viability analysis subsequently indicated that mean overall reductions of 75% and 74% were obtained for the analysed indicator organisms (*E. coli* and enterococci spp.) and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* spp. oocysts) in the Prototype I and II solar reactors, respectively. The large-volume batch solar reactor prototypes could thus effectively provide four (88 L Prototype II) to seven (144 L Prototype I) people on a daily basis with the basic water requirement for human activities (20 L). Additionally, a generic Water Safety Plan was developed to aid practitioners in identifying risks and implement remedial actions in this type of installation in order to ensure the safety of the treated water.

Keywords: Rainwater harvesting; Large-volume SODIS reactors; EMA-qPCR; rainwater quality; water scarcity

1. Introduction

The Global Risks Report released for 2019 listed water crises as one of the top ten risks in terms of likelihood (9th overall; very likely to occur) and impact (4th overall; severe impact) (World Economic Forum, 2019). The probability of a water crisis risk in sub-Saharan Africa is significantly increased as a high proportion of the population reside in urban informal settlements (densely populated areas with inadequate water and municipal services) and rural areas, with limited access to a safe water supply and waste disposal and sanitation infrastructure (Dos Santos et al., 2017). However, as highlighted by Gwenzi and Nyamadzawo (2014) and Emenike et al. (2017), rainwater is considered an underutilised water source in sub-Saharan Africa and may serve as an effective reserve to improve and encourage equity in water access. Roof-harvested rainwater (RHRW) can however, be contaminated with various chemicals and microorganisms, which may limit its use as a potable water source (Hamilton et al., 2019). While the chemical pollutants have not been directly associated with the incidence of disease, organic debris, faecal matter from animals that have access to the catchment surface and bioaerosol particles, have been identified as the primary sources of microbial contaminants such as *Legionella*, *Klebsiella*, *Pseudomonas* and *Cryptosporidium* (Hamilton et al., 2019).

Treatment strategies that may be implemented to improve the quality of rainwater include the utilisation of gutter screens or first-flush diverters for the prevention of contaminant entry into the collection tank or post-collection treatment [chemical (e.g. chlorination) and physical treatments (e.g. filtration, solar disinfection (SODIS) and thermal disinfection)] (Hamilton et al., 2019; Senevirathna et al., 2019). Although various chemical and physical treatment technologies have been investigated, SODIS is considered a cost-effective treatment method and is recommended by the World Health Organisation (WHO) for the effective reduction of microbial contamination in water sources (Ubomba-Jaswa et al., 2010). In its simplest form, SODIS entails filling a transparent container [usually a 2 L polyethylene-terephthalate (PET) bottle] with contaminated water and exposing the bottle to direct sunlight for six to eight hours to allow ultraviolet (UV) radiation and solar-mild heat to inactivate microbial contaminants (McGuigan et al., 2012). Ultraviolet radiation directly inactivates the microbial contaminants by damaging nucleic acids and leads to the formation of reactive oxygen species (ROS), which react and damage proteins, nucleic acids and membrane lipids (Nelson et al., 2018). The water temperature will also increase as water molecules absorb the UV radiation, which leads to cell membrane damage (≥ 45 °C) (McGuigan et al., 2012). The major drawbacks associated with this technique are the small volumes of water that can effectively be treated (2 to 5 L) and decreased efficiency during overcast weather conditions (requiring up to 48 hours of treatment). Increases in treatment volume and efficiency may then be obtained by employing various modifications (SODIS enhancement technologies) such as solar mirrors (concentrates UV radiation) and larger reactor tubes (increase treatment volume) (Ubomba-Jaswa et al., 2010; McGuigan et al., 2012).

As part of the European Union (EU) Horizon 2020 WATERSPOUTT project (grant agreement no. 688928), Martínez-García et al. (Unpublished results A) investigated various enhancement technologies that may cost-effectively allow for larger volumes of water to be treated using SODIS. Results from the study indicated that the use of a static batch reactor system employing V-trough solar mirrors allowed for the effective treatment of a larger volume (68% more) of water compared to the compound parabolic collector (CPC)-type solar mirrors under the same solar exposure conditions. In a follow-up study, the same research group designed two large-volume batch solar reactor prototypes (static batch systems with 88 L and 140 L treatment volumes, respectively), where multiple poly(methyl methacrylate) (PMMA) reactor tubes were positioned in the centre of V-trough solar mirrors (Martínez-García et al., Unpublished results B). Preliminary assessment of the solar reactor prototypes, using spiked synthetic rainwater samples and culture-based analysis, indicated that a ≥ 6 log removal efficiency was obtained for *Escherichia coli* (*E. coli*) and *Salmonella enteriditis* after 1.5 hour natural sunlight exposure, while a 2 hour sunlight exposure was required to achieve the same log reduction for *Enterococcus faecalis* and *Pseudomonas* *aeruginosa* (*P. aeruginosa*). Although the preliminary assessment of the solar reactor prototypes display promise in treating rainwater, it is crucial that these systems be assessed on-site in the target communities, i.e. rural areas and urban informal settlements. This will allow for a more comprehensive indication as to whether these reactors may serve as a sustainable solution in providing communities with a safe alternative water source.

The primary aim of the current study was thus to assess the efficiency of the two newly designed WATERSPOUTT large-volume batch solar reactor prototypes (Martínez-García et al., Unpublished results B) for the treatment of RHRW on-site in a local informal settlement (140 L Prototype I) and a rural farming community (88 L Prototype II). The chemical quality of the RHRW before and after solar reactor treatment was routinely assessed by monitoring various physico-chemical parameters (e.g. temperature, pH, and turbidity), anions and cations. Additionally, the removal of traditional indicator organisms (*E. coli*, total and faecal coliforms, enterococci and heterotrophic bacteria) and selected opportunistic pathogens (*Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp.), was assessed using culture-based analysis. Ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) assays were also used to monitor the reduction efficiency of indicator organisms (*E. coli* and enterococci) and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.) (Fields et al., 2002; Eng et al., 2015; Clements et al., 2019; Strauss et al., 2019), while propidium monoazide (PMA) qPCR assays were used to monitor *Cryptosporidium* spp. oocyst reductions. A Water Safety Plan (WSP) outlining guidelines for the use of rainwater harvesting combined with solar reactor treatment was also implemented, as this may aid in ensuring the safety of the treated RHRW.

2. Materials and methods

2.1 Description of the large-volume batch solar reactor prototypes and sampling sites

Two large-volume batch solar reactor prototypes were designed and constructed as part of the WATERSPOUTT project (grant agreement no. 688928) for implementation in South Africa and Uganda, with the current study focusing on the application of these systems in field trials in South Africa. The Prototype I solar reactor (140 L treatment volume) was installed in Enkanini informal settlement (Site 1; GPS coordinates: 33°55'28.1"S 18°50'35.8"E) during July 2018 and consisted of three PMMA reactor tubes (200 mm diameter) that were positioned in the centre of a V-trough solar mirror (constructed from anodized aluminium). The reactor tubes were positioned at a 34° angle (equal to the local latitude to optimise the average annual solar UV irradiance input to the solar reactor) and were inter-connected by UV-A transparent PMMA tubing (Fig. 1.A). The Prototype II solar reactor (88 L treatment volume) was installed next to a local church building in the Skoolplaas farming community (Site 2; GPS coordinates: 33°56'38.5"S 18°46'26.3"E) during July 2018 and consisted of the same materials and design as Prototype I, with the exception that eight PMMA tubes (100 mm diameter) were substituted for the three 200 mm diameter tubes used in the Prototype I system (Fig. 1.B). Additionally, as space was available between the gutter system and the rainwater harvesting (RWH) tank at site 2, a first-flush (FF) diverter with built-in leaf and insect screens (Superhead® rainwater filter) was installed to redirect the initial roof run-off during a rain event (Fig. 1.B). A detailed description of the sampling sites, system installation and schematic diagrams of the large-volume batch solar reactors is outlined in Appendix A, while additional information regarding the working mechanism of the large-volume batch solar reactors is outlined in Appendix B.

2.2 Ethical clearance and sample collection

Exemption from ethical clearance was obtained from the Research Ethics Committee (Humanities) Stellenbosch University (Ethics Reference no.: SU-HSD-004624), as the participating households were instructed to only use the treated water for domestic uses and not for drinking purposes.

For the microbial and chemical analysis of the water produced by the solar reactor prototypes (Fig. 1), an untreated 10 L sample was collected directly from the RWH tank at each site [hereafter referred to as Tank 1 (Site 1) and Tank 2-FF (Site 2)] on the morning of a sampling event. The respective solar reactor prototypes at each site were then immediately filled with tank water from the RWH tanks and exposed to direct sunlight for 6 hours (sampling sessions 1 to 8) or 8 hours (sampling sessions 9 to 18). Following the completion of the solar exposure, 10 L of each treated sample was collected directly from the solar reactors [hereafter referred to as Prototype I (Site 1) and Prototype II (Site 2)]. Based on the availability of rainwater in the RWH tanks, 15 sampling sessions were conducted at site 1 (n = 30; August 2018 to March 2019), while 18 sampling sessions were conducted at site 2 (n = 36; August 2018 to April 2019). For ease of presentation, sampling sessions 1 to 18 are designated as #1 (sampling session 1), #2 (sampling session 2), etc., throughout the manuscript.

The temperature, pH, electrical conductivity and total dissolved solids present in all water samples were measured using a hand-held Milwaukee Instruments MI806 meter (Spraytech, South Africa), while the dissolved oxygen was measured using a Milwaukee Instruments M600 meter (Spraytech, South Africa). Rainfall and daily ambient temperature data for the study period was obtained from the South African Weather Services, while solar irradiance data [mean ambient UV-A and UV-B radiation] was obtained from the Stellenbosch Weather Services [Stellenbosch University, Faculty of Engineering (http://weather.sun.ac.za/)].

2.3 Chemical analysis

The chemical quality of the untreated and solar reactor treated tank water samples was determined by monitoring cation and anion concentrations and measuring sample turbidity (Strauss et al. 2018). Briefly, for cation analysis, 50 mL Falcon™ high-clarity polypropylene tubes (Corning Life Sciences, USA) and polyethylene caps were pre-treated with 1% nitric acid before sample collection. Following sample collection, the concentration of 25 cations (outlined in Table A.3 of the supplementary information) were determined after acidification (1% ultrapure nitric acid) using inductively coupled plasma mass spectrometry (Agilent 7700 ICP-MS) by the Central Analytical Facility (CAF) at Stellenbosch University. One litre water samples were collected for anion and turbidity analyses (outlined in Table A.3 of the supplementary information) and processed by Bemlab Laboratories (Cape Town, South Africa) using a Thermo Scientific Gallery™ Automated Photometric Analyser. All samples (n = 66) were monitored for cations, while representative samples (n = 22; #1, #7, #10, #12, #15 and #18) were monitored for anions and turbidity. Representative samples were analysed for anions and turbidity as previous research conducted by members of our research group indicated that anion concentrations in rainwater collected from the region (Stellenbosch), adhered to drinking water standards (Dobrowsky et al., 2015; Reyneke et al., 2016; 2018; Strauss et al., 2016; 2018). Similarly, the rainwater samples were also found to have low levels of turbidity [<1.00 Nephelometric Turbidity Units (NTU)] which adhered to drinking water standards (Strauss et al. 2016; 2018).

2.4 Culturing of indicator organisms and opportunistic pathogens

The microbial quality of the tank water samples collected from sites 1 and 2 was monitored before (untreated) and after solar reactor treatment using various culture-based analyses. *Escherichia coli* and total coliforms were enumerated simultaneously using membrane filtration as described by Dobrowsky et al. (2015). Briefly, a total volume of 100 mL (undiluted, 10^{-1} and 10^{-2}) was filtered through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (70 kPa). The filters were then placed onto Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) and were incubated at 35 ± 2 °C for 18 - 24 hrs. In order to enumerate enterococci, 100 µL of an undiluted sample was spread plated onto Slanetz and Bartley

Agar (Oxoid), with the plates incubated for 44 – 48 hrs at 36 ± 2 °C (Strauss et al., 2016). In order to enumerate faecal coliforms (FC), 100 µL of an undiluted sample was spread plated onto m-FC Agar (Biolab, Merck, Wadeville, South Africa), with the plates incubated for 44 – 48 hrs at 35 ± 2 °C (Strauss et al., 2016). For the enumeration of the heterotrophic plate count/bacteria (HPC), a serial dilution (10^{-1}–10^{-3}) was prepared for each sample and by use of the spread plate method 100 µL of an undiluted sample and each dilution (10^{-1}–10^{-3}) was plated onto Luria Bertani (LB) agar (Biolab), with the plates incubated at 37 °C for up to four days. For the treated samples (Prototypes I and II) where the HPC were reduced to below the detection limit [BDL; < 1 colony forming units (CFU)/1 mL], the potential regrowth of bacteria was monitored. Briefly, 20 mL of each treated sample was stored in a sterile McCartney bottle at room temperature and 100 µL of the treated water was spread plated onto LB agar (Biolab, Merck) every 24 hours for a period of 2 days. The plates were then incubated at 37 °C. Additionally, *Klebsiella* spp. (HiCrome™ *Klebsiella* Selective Agar; Sigma-Aldrich, St Louis, MO), *Pseudomonas* spp. (*Pseudomonas* Isolation Agar; Sigma-Aldrich) and *Salmonella* spp. (*Salmonella*-*Shigella* Agar; Oxoid) were enumerated as outlined in Clements et al. (2019) by spread plating 100 µL of an undiluted sample onto the respective media and incubating the plates at 37 °C for 18 to 24 hours. Additionally, coliphages were enumerated as outlined by Baker et al. (2003) using *E. coli* ATCC 13706 as the target bacterial host. All culture-based analyses were performed in duplicate.

2.5 Tank water concentration, viability treatment and DNA extraction

The concentration of 1 L (Site 1) and 2 L (Site 2) samples, EMA treatment and subsequent DNA extractions were performed for each of the samples collected before and after solar reactor treatment as outlined in Reyneke et al. (2016). An increased sample volume was processed for site 2 in order to obtain sufficient DNA for the subsequent molecular-based analysis. For the molecular quantification of *Cryptosporidium* spp. within the collected samples, the same methodology was repeated with the exception that a PMA treatment as described by Alonso et al. (2014) was followed.

2.6 Molecular-based enumeration of indicator organisms and opportunistic pathogens

Quantitative PCR was performed in order to quantify *E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp. in all of the collected tank water samples, while *Cryptosporidium* spp. oocysts were quantified in the samples collected from #9 to #15 and #9 to #18 for sites 1 and 2, respectively (an insufficient volume of water was available for #1 to #8 for the additional tank water concentration and PMA treatment required for *Cryptosporidium* spp. oocyst detection and quantification). All qPCR assays were conducted using a LightCycler® 96 (Roche Diagnostics, Risch-Rotkreuz, Switzerland) instrument in combination with the FastStart Essential DNA Green Master Mix (Roche Diagnostics) as outlined in Reyneke et al. (2017), with the primer pairs and cycling parameters presented in Table A.1. Standard curves for the respective qPCR assays were generated using the methodology outlined in Reyneke et al. (2017), while the qPCR performance characteristics of the various assays were analysed using the Roche LightCycler® 96 Software Version 1.1. Furthermore, to compensate for the different sample volumes used per site for rainwater concentration [1 L (Site 1) and 2 L (Site 2)] the gene copies detected in the samples utilising the qPCR assays were converted to gene copies per 100 mL of the original tank water sample as outlined by Waso et al. (2018). The gene copy numbers (gene copies/100 mL) were then converted to cell equivalents (cells or oocysts/100 mL) by utilising the number of copies of the target gene present within the target host (Table A.1). All final concentrations for qPCR analyses are thus presented as equivalent cells or oocysts/100 mL original tank water sample.

2.7 Maintenance of prototype reactors and water safety plan

Following the system installations, workshops were conducted within the respective communities to outline the principle of rainwater harvesting, the working mechanism and operational maintenance of the solar reactors (Fig. A.4). Information on the domestic activities (i.e. laundry, cleaning, washing, etc.) the treated rainwater could be used for was also provided (Fig. A.5).

As outlined by the WHO (2004), the most efficient way of consistently ensuring the safety of a drinking water supply is through the utilisation of a WSP (Appendix B), which is defined as a risk assessment and management approach that monitors the entire water supply process (e.g. collection of RHRW to utilisation of treated water by the consumer). The first step in the development of the WSP was to develop a simplified guide to RWH and the use of the solar reactor prototypes that would provide the end-users with a basic description of the technology and guidelines for the implementation and maintenance of the system (Appendix B). This was achieved by identifying all potential hazards and hazardous events that may influence the quality of rainwater during the harvesting, storage and treatment process (Appendix B), using published literature and personal observations at the respective study sites, during the study period. Thereafter, various maintenance and remedial actions were identified to prevent certain water safety hazards (e.g. prevent organic debris from entering the storage tank) or to implement after a hazardous event occurred (e.g. control measure failed and organic debris washed into the storage tank) (Appendix B). Following the identification of the potential hazards, a risk assessment matrix (Appendix C) was compiled that would enable the risk characterisation associated with each hazard/hazardous event and enable the assessment of the various control measures (e.g. maintenance strategies, use of a first-flush diverter system etc.) in eliminating or minimising the identified water safety hazards.

2.8 Statistical analysis

Statistical analyses were conducted utilising either RStudio (version 1.0.153) or Minitab19. Shapiro-Wilk tests were performed in order to determine whether the data was evenly or non-evenly distributed. Overall differences in sample composition between site 1 and site 2 and the untreated (Tank 1 and Tank 2) and solar reactor treated (Prototype I and II) tank water samples was then determined by evaluating all measured physico-chemical, chemical and microbial parameters using either the parametric paired *t*-test or the non-parametric Wilcoxon test (significant when $p < 0.05$). Principle component analysis (PCA) was then used to visualise the correlations between the measured cations at both sites and identify which cations primarily influenced the sample composition at each site.

3. Results and Discussion

3.1 Physico-chemical properties and chemical analysis of the collected tank water samples

The daily rainfall and ambient temperatures recorded throughout the 2018/2019 research period as well as the sampling sessions for each site are depicted in Fig. A.6. A total rainfall of 431.4 mm was recorded during July 2018 to September 2018 (high rainfall period), while 183.8 mm was recorded during October 2018 to January 2019 (medium rainfall period). The rainfall then decreased to 146.2 mm during February to April 2019 (low rainfall period). The mean ambient UV-A radiation at both sampling sites ranged from 7.16 W/m^2 (12/09/2018) to 31.29 W/m^2 (14/01/2019), while the mean ambient UV-B radiation ranged from 1.33 W/m^2 (12/09/2018) to 4.63 W/m^2 (14/01/2019) (Table A.2). The untreated tank water temperature at site 1 (Tank 1) ranged from 9.0 °C (02/08/2018 and 15/08/2018) to 24.0 °C (28/01/2019), with a mean temperature of 16.3 °C recorded for all sampling days, while the tank water temperature in the samples collected from the Prototype I solar reactor ranged from 15.5 °C (12/09/2018) to 45.0 °C (28/01/2019) (mean 28.9 °C) (Table A.2). Similarly, the untreated tank water temperature at site 2 (Tank 2-FF) ranged from 10.0 °C (15/08/2018) to 26.0 °C (25/10/2018) (mean 18.1 °C), while the tank water temperature in the samples collected from the Prototype II solar reactor ranged from 18.0 °C (12/09/2018) to 46.5 °C (28/01/2019) (mean 32.6 °C) (Table A.2).

All measured physico-chemical parameters (pH, turbidity, electrical conductivity, total dissolved solids and dissolved oxygen) in the collected untreated and prototype treated rainwater samples adhered to the drinking water guideline limits of the South African

Department of Water Affairs and Forestry (DWAF) (DWAF, 1996), South African National Standards (SANS) 241 [South African Bureau of Standards (SABS), 2005], Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and WHO (2017), with no significant difference ($p > 0.05$) observed for the data collected for the untreated and treated (Tank 1 and Prototype I; Tank 2-FF and Prototype II) tank water samples or between sites 1 and 2 (Tank 1 and 2-FF) (Table A.3).

Results for the chemical analyses of the untreated (Tank 1 and Tank 2-FF) and treated (Prototype I and Prototype II) tank water samples collected from sites 1 and 2, indicated that all anions and cations (Table A.3) were within the respective drinking water guideline limits [DWAF, 1996; SANS 241 (SABS, 2005); ADWG (NHMRC and NRMMC, 2011); WHO, 2017], with the exception of the mean zinc (Zn) concentration recorded in the samples collected from site 1 [Tank 1 (mean of 3044 µg/L) and Prototype I (mean of 3061 µg/L)]; which exceeded (albeit not significantly) the DWAF (1996) and ADWG (NHMRC and NRMMC, 2011) limit of 3000 µg/L. However, these samples were within the 5000 µg/L SANS 241 (SABS, 2005) limit. The increased Zn concentrations recorded at site 1 (Tank 1 and Prototype I), in comparison to site 2 (Tank 2-FF and Prototype II), may primarily be attributed to the Zn metal sheeting roofing material used to construct the catchment system, as the leaching of metals from metal roofing materials (corrosion during rain events and continuous exposure to sunlight) have been reported to be a major contributor of metal ions in rainwater (Chang et al., 2004; Reyneke et al., 2018). It should be noted, that while the catchment system at site 2 was also constructed from Zn sheeting roofing material, the entire surface of the catchment system was painted with a weather resistant roof paint (personal communication) which may have limited the leaching of metal ions into the rainwater. Additionally, the first-flush diverter connected to the rainwater tank at site 2 (Tank 2-FF) may have improved the physico-chemical quality of the tank water samples. First-flush diverter systems act as a pre-treatment barrier by redirecting the initial roof run-off water (at the start of a rain event), which is thought to contain the highest concentration of pollutants (Sánchez et al., 2015). Gikas and Tsihrintzis (2012) compared the quality of RHRW collected in the flush pipe of first-flush diverter systems, with the RHRW entering the collection tanks (RWH tanks) and reported that all measured mean anion and cation concentrations were higher in the collected first-flush samples. The authors concluded that the diversion of the first-flush roof run-off away from the collection tanks improved the physico-chemical quality of the RHRW.

As no significant difference was obtained when comparing the anion and cation concentrations (Table A.3) recorded in the untreated tank water samples to the treated tank water samples (Tank 1 vs Prototype I, Tank 2-FF vs Prototype II) and the tank water samples from each site clustered together (Fig. 2), it was concluded that the solar reactor prototypes (system components and the treatment mechanism) did not influence the chemical quality of the tank water samples.

3.2 Removal efficiency of indicator bacteria and opportunistic pathogens

3.2.1 Culture-based analysis

For the untreated tank water samples collected from site 1 (Tank 1; n = 15), the *E. coli*, faecal coliform, total coliform, enterococci and HPC concentrations exceeded the respective drinking water guideline limits in 67%, 73%, 100%, 20% and 100% of the samples, respectively (Table 1). Analysis of the corresponding treated samples (Prototype I; n = 15) indicated that the *E. coli* (> 0.78 log reduction), enterococci (> 3.48 log reduction) and faecal coliform (> 4.08 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all the collected samples. Total coliforms were reduced to BDL in 63% of the treated samples collected following a 6 hour solar exposure (# 1-8) (> 3.94 log reduction), with a mean of 55 CFU/100 mL detected in the samples (37%) where total coliform counts above the standard were detected. An increase in solar exposure to 8 hours (# 9-15) resulted in an increased treatment efficiency, as total coliforms were reduced to within the 5 CFU/100 mL DWAF (1996) and 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits in 100% of the treated samples (4.66 log reduction). For the HPC analysis, 38% of the treated samples were reduced to within the drinking water guideline limit of 1.0×10^4 CFU/100 mL (1.71 log reduction) after a 6 hour solar exposure [mean of 2.4×10^4 CFU/100 mL detected in the remaining 63% samples (1.21 log reduction)], while 57% of the treated samples were reduced to below the guideline limit (2.08 log reduction) after an 8 hour solar exposure [mean of 2.7×10^4 CFU/100 mL detected in the remaining 43% of samples (1.01 log reduction)] (Fig. A.8).

For the untreated tank water samples collected from site 2 (Tank 2-FF; n = 18), the *E. coli*, faecal coliform, total coliform, enterococci and HPC concentrations exceeded the respective drinking water guideline limits in 56%, 22%, 100%, 28% and 100% of the samples, respectively (Table 1). Analysis of the corresponding treated samples (Prototype II; n = 18) indicated that the *E. coli* (> 0.48 log reduction), enterococci (> 3.34 log reduction) and faecal coliform (> 3.04 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all collected samples, while total coliforms were reduced to below the 5 CFU/100 mL DWAF (1996) and 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits (3.85 log reduction). Heterotrophic bacteria were then reduced to below the 1.0×10^4 CFU/100 mL DWAF (1996) drinking water guideline limit in 88% of the treated samples (mean of 4.6×10^3 CFU/100 mL recorded) after a 6 hour solar exposure (# 1-8) (2.11 log reduction), with a mean of 1.8×10^4 CFU/100 mL detected in the samples (12%) where HPC concentrations above the standard were detected. In comparison, 100% of the treated samples were reduced to below the 1.0×10^4 CFU/100 mL drinking water guideline limit after an 8 hour solar exposure (# 9-18) (≥ 2.02 log reduction; Fig. A.8).

Klebsiella spp. were detected in 100% (mean concentration of 1.9×10^4 CFU/100 mL) and *Salmonella* spp. in 60% (mean concentration of 6.3×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 1 (Tank 1); however, both organisms were reduced to BDL (> 4.28 and > 3.8 log reduction, respectively) following treatment using the Prototype I solar reactor (Table 1). *Klebsiella* spp. were also detected in 17% (mean concentration of 8.0×10^2 CFU/100 mL) and *Salmonella* spp. in 6% (mean concentration of 1.0×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 2 (Tank 2-FF), with both organisms reduced to BDL (> 2.9 and > 3 log reduction, respectively) following treatment using the Prototype II solar reactor (Table 1). *Pseudomonas* spp. and coliphages were not detected in any of the rainwater samples collected from sites 1 and 2.

Although numerous studies have investigated the use of SODIS to treat contaminated water, varying degrees of treatment efficiency (0.46 to > 6 log reductions in bacteria) have been reported depending on experimental design (McGuigan et al., 2012; Hamilton et al., 2019). However, a limitation of SODIS which has consistently been highlighted by these investigators is the small treatment volume (2 to 5 L). Ubomba-Jaswa et al. (2010) investigated the use of a 25 L SODIS reactor (methacrylate tube) situated inside a CPC and reported on the complete inactivation of *E. coli*, even during unfavourable weather conditions (cloudy with low solar intensity). Martínez-García et al. (Unpublished results B) then expanded on this research and investigated cost-effective SODIS enhancement strategies that would enable the treatment of larger volumes of water (32 L and 54 L), with the results obtained leading to the design of the large-volume batch solar reactor prototypes (Prototype I and II) assessed in the current study. The treatment efficiency of the Prototype I and II solar reactors was also assessed by Martínez-García et al. (Unpublished results B) under controlled conditions, by spiking synthetic rainwater with laboratory strains of *E. coli*, enterococci, *Salmonella* and *Pseudomonas* (10^5 – 10^6 CFU/mL bacterial cells) using a 6 hour solar exposure treatment time. A ≥ 6 log reduction of all the test bacteria was obtained, with the system classified as "highly protective (≥ 4 log reduction)" against bacteria according to the WHO (2016) household water treatment technology performance criteria. In comparison, results from the current study, for both solar reactor prototypes, during a 6 hour solar exposure treatment, indicated that a ≥ 2.54 log reduction was obtained when monitoring the removal of enterococci, faecal and total coliforms, while mean log reductions of ≥ 1.21 log were obtained for the removal of HPC. Based on these results, the 6 hour solar exposure treatment with the prototypes in field trials failed to meet the ≥ 2 log removal required for a "protective" classification against bacteria. The Martínez-García et al.

(Unpublished results B) study was however, conducted in a hot arid climate (Tabernas Desert, Almería, Southern Spain) with a mean UV radiation of 28.31 W/m^2/h recorded during the 6 hour treatment trials, while the field trials of the systems in the current study were conducted in a moderate Mediterranean climate (Stellenbosch, Western Cape, South Africa), where a mean UV radiation of 20.82 W/m^2/h was recorded during the 6 hour treatment trials (Table A.2).

The treatment time in the current study was subsequently increased to 8 hours (Site 1: #9-15; Site 2: #9-18) in order to increase the overall UV dose (mean UV radiation of 24.72 W/m^2/h was recorded from #9-18). For both prototypes a ≥ 3.44 log reduction was subsequently obtained when monitoring the removal of enterococci, faecal and total coliforms, while the mean log reductions for the removal of HPC increased to ≥ 2.02 log. Based on the observed treatment efficiencies obtained using the Prototype I and II solar reactors in the current study (8 hour treatment), the prototypes may be classified as "protective (≥ 2 log reduction)", for the removal of bacteria in the tank water (WHO, 2016). More importantly, culture-based analysis indicated that both treatment systems were able to produce water that adhered to the microbial parameters as stipulated in the respective drinking water guidelines [DWAF, 1996; SANS 241 (SABS, 2005); ADWG (NHMRC and NRMMC, 2011); WHO, 2017], with lower indicator organism counts recorded in the tank water samples collected from site 2, where the first-flush diverter system was installed. The treated water collected from the large-volume batch solar reactor prototypes could however, only be stored for a maximum of 24 hours, as microbial regrowth occurred after this point (2.0×10^3 CFU/100 mL to 1.80×10^4 CFU/100 mL detected after 24 hours).

3.2.2 Molecular-based analysis

The performance characteristics of the respective qPCR assays are provided in Table A.4. Results obtained using EMA-qPCR indicated that an overall mean decrease of 83.76% (0.79 log reduction) in intact *E. coli* cells was recorded after treatment using Prototype I, while an overall mean decrease of 82.76% (0.76 log reduction) was recorded after treatment for

Prototype II (Fig. 3). Similarly, intact enterococci cells decreased by a mean of 91.68% (1.08 log reduction) after treatment using Prototype I, while an 84.89% (0.82 log reduction) mean decrease was recorded after treatment using Prototype II (Fig. 3). In comparison, quantification of intact *Klebsiella* cells indicated that this genus was more resistant to the solar reactor treatment as mean decreases of 62.44% (0.43 log reduction) and 60.42% (0.40 log reduction) were recorded after treatment using Prototype I and II, respectively (Fig. 3). Similarly, intact *Legionella* cells decreased by 68.61% (0.50 log reduction) after treatment using Prototype I and by 63.77% (0.44 log reduction) after treatment using Prototype II (Fig. 3). Overall mean decreases in intact *Pseudomonas* cells of 79.09% (0.68 log reduction) and 87.50% (0.90 log reduction) were recorded after treatment using Prototype I and II, respectively, while *Salmonella* cells decreased by 78.36% (0.66 log reduction) after treatment using Prototype I and 67.82% (0.49 log reduction) after treatment with Prototype II (Fig. 3). Lastly, PMA-qPCR analysis indicated that *Cryptosporidium* spp. oocysts decreased by 57.14% (0.62 log reduction) after treatment using Prototype I, while a mean decrease of 73.81% (0.58 log reduction) was recorded after treatment using Prototype II (Fig. 3).

For the monitored indicator organisms and opportunistic pathogens, EMA-qPCR (*E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.) and PMA-qPCR (*Cryptosporidium* spp. oocysts) analysis indicated that a mean overall reduction of 74.43% was obtained following treatment for both the Prototype I and II solar reactors. This discrepancy in the observed treatment efficiency in comparison to the results obtained using culture-based analysis, may be attributed to EMA-qPCR and PMA-qPCR detecting viable but non culturable (VBNC) cells within the water samples (Fittipaldi et al., 2012; Mansi et al., 2014). It has been reported that certain opportunistic pathogens (e.g. *Legionella pneumophila* and *P. aeruginosa*) can enter a VBNC state in which they are not detectable using standard culture-based analysis but are still viable and retain their virulence (Mansi et al., 2014). Moreover, these VBNC microorganisms may regain their ability to be cultured under favourable conditions, which corresponds to the observed bacterial regrowth observed after 24 hours (culture-based analysis). Strauss et al. (2019) then applied Illumina next-generation sequencing coupled with EMA viability treatment to identify the primary pathogenic or opportunistic pathogenic genera, capable of surviving SODIS-CPC treatment in a 10.6 L CPC-reactor (Strauss et al., 2019). Results from the study indicated that intact and potentially viable bacterial cells belonging to 11 different bacterial genera (e.g. *Acinetobacter*, *Campylobacter*, *Legionella*, *Mycobacterium* and *Pseudomonas* amongst others) were detected in the SODIS-CPC treated tank water. Thus while the use of indicator bacteria (culture-based analysis) has become routine when monitoring water quality, it should be noted that there is a poor correlation between the presence of faecal indicators and potential pathogenic bacteria (Ahmed et al., 2008). Monitoring for the removal of potentially pathogenic microorganisms which may have entered a VBNC state following water treatment is thus essential as these VBNC bacteria still pose a health risk as they are potentially infectious (Mansi et al., 2014).

While the survival of the *Cryptosporidium* spp. oocysts after SODIS treatment using the solar reactor prototypes, may be attributed to the resilient nature of the oocyst wall (Hamilton et al., 2018), the ability of the opportunistic pathogenic bacteria (*Pseudomonas* spp., *Salmonella* spp., *Legionella* spp. and *Klebsiella* spp.) to survive large-volume solar-based disinfection strategies has been attributed to their ability to initiate various stress-response mechanisms and switch to a more tolerant phenotype upon exposure to environmental stressors, such as temperature and UV exposure (Jones, 1997; Fux et al., 2005). These stress-responses may include the production of heat shock proteins and the initiation of DNA repair mechanisms, amongst others (Fields et al., 2002; Breidenstein et al., 2011). For example, Srivastava et al. (2008) indicated that the overexpression of the sigma factor *algT*, protects *Pseudomonas* spp. from heat stress and allows these organisms to persist during unfavourable conditions, while DNA repair mechanisms may be initiated in response to UV-induced DNA damage, through the activation of the SOS-regulon (upregulation of *recA* and *lexA* genes) or the photolyase enzyme (Zenoff et al., 2006). Similarly, Bojer et al. (2010) attributed the heat resistance of *K. pneumoniae* to the *clpK* genetic marker, which has been shown to correlate positively with thermotolerant phenotypes observed among clinical *Klebsiella* isolates. Microorganisms have also been reported to produce pigments or structures that may enable their survival under unfavourable conditions, as has been reported for *P. aeruginosa*, where the production of pyocyanin has been hypothesised to protect *P. aeruginosa* from oxidative stress (inactivation mechanism of SODIS) (Hendiani et al., 2019). It is thus evident that microorganisms may employ numerous strategies to survive disinfection treatment and that additional treatment barriers may be required to reduce the survival of these target pathogens within water treatment systems. These strategies may include the addition of a cost-effective filtration system as a pre-treatment strategy to reduce microbial load entering the large-volume batch solar reactor prototypes (Hamilton et al., 2019).

3.3 Water safety plan, end-user engagement and operational sustainability of the systems

As numerous factors may influence the quality of RHRW during the harvesting and/or treatment process, a WSP (Appendix B) for the utilisation of rainwater harvesting in combination with the large-volume batch solar reactor prototypes was developed. As the WSP was developed concurrently with the monitoring of the large-volume batch solar reactor prototypes during the field trials, the effectiveness of the various control measures was assessed by comparing site 1 with site 2, as these sites were located in two distinct settings that could be influenced by different anthropogenic activities and potential pollution sources as outlined in Appendix A.

The application of the WSP to characterise the risk associated with RHRW collected at sites 1 and 2, indicated that the external hazards at site 1 (informal settlement) posed a greater risk of contamination. The increased risk was primarily attributed to the influence of potential pollution sources present near the catchment system (e.g. garbage disposal site, surface run-off), tree branches obstructing a section of the conveyance system, organic debris (e.g. dust/soil dispersed from the dirt pathway, leaves from the tree) within the conveyance system and corrosion of the metal sheeting catchment system.

Correspondingly, chemical and microbial analysis of the untreated tank water samples collected from sites 1 and 2 revealed that the untreated tank water collected from site 1 had higher levels of chemical contaminants (e.g. cations) and microbial contaminants in comparison to site 2. For example, the concentration of HPC was 0.72 log [3.50×10^5 CFU/100 mL (Tank 1) vs 6.90×10^4 CFU/100 mL (Tank 2-FF)] greater in the untreated tank water samples from site 1 (Tank 1), in comparison to site 2 (Tank 2-FF).

The improved tank water quality at site 2 may also be attributed to the efficiency of the implemented control measures at this site. The catchment surface at site 2 was painted with a weather resistant roof paint (personal communication) that may have reduced the leaching of metal contaminants into the collected tank water. Additionally, due to space availability a first-flush diverter was connected between the catchment system and Tank 2-FF, which served as a control measure to reduce the introduction of organic debris into the collection tank. However, the efficiency of a first-flush diverter is dependent on the maintenance of the system, which entailed cleaning/emptying the first-flush diverter after each rain event. The quality of RHRW collected from site 1 may then be improved by removing the obstructing tree branches (source of organic debris), implementing a regular gutter cleaning regime, installing a gutter screen at the inlet of the RWH tank (due to space limitation a first-flush diverter could not be connected to the current catchment system) and replacing the corroded metal sheeting on the catchment system or painting the catchment system with a weather resistant roof paint.

As previously indicated, workshops were conducted with participating households within the respective communities to outline the operational maintenance of the large-volume batch solar reactor prototypes and rainwater harvesting systems (Fig. A.4 and Fig. A.5). Subsequent monitoring of the operational sustainability of the solar reactor prototypes at both sites indicated that system maintenance was limited to cleaning the surface of the PMMA reactor tubes (prevent dust accumulation that will influence UV transmittance), with no system components needing replacement during the study period. The potential degradation (leaching) of the PMMA reactor tubing is however, being investigated by members of the WATERSPOUTT research consortium. The robustness and cost of system components should therefore be taken into consideration when designing water treatment systems for use in rural areas and informal settlements, where replacement components may not be readily available (Mwabi et al., 2011; McGuigan et al., 2012). A preliminary cost analysis for the solar reactor prototypes has been included in Appendix A, with the cost (US$/L) compared to the costs associated with other household drinking water treatment systems (Table A.5). During the study period, households who had access to the treated tank water (Prototype I and II) at sites 1 (13 households) and site 2 (5 households), primarily reported using the treated tank water for domestic activities such as cleaning of their homes, laundry and washing.

As noted by Mahmud et al. (2007), the aim of a WSP for small community water supplies should be to achieve an overall and sustained reduction in microbial contaminants/sanitary risks, rather than aim for the complete removal of microbial contaminants. The WSP outlined in the current study thus serves to reduce the contamination of RHRW by reducing "preventable contaminant entry" (e.g. organic debris and faecal matter containing an increased microbial load from washing into the storage tank) into the storage tank, whereafter treatment with the large-volume batch solar reactor prototypes may further reduce the microbial contaminants to within drinking water standards.

4. Conclusions

The physico-chemical and chemical quality of the Tank 1 and 2-FF and Prototype I and II treated rainwater samples adhered to the respective drinking water guidelines, with an improvement in quality observed at site 2 where the first-flush diverter was installed. Lower indicator bacterial counts were also recorded in the tank water samples collected from site 2 (Tank 2-FF and Prototype II) where the first-flush diverter was installed and fewer hazards were identified that may influence the tank water quality (WSP), in comparison to site 1 (Tank 1 and Prototype I). The installation of a first-flush diverter system may thus serve as an inexpensive pre-treatment strategy that may improve the overall quality of RHRW, while the establishment of a WSP may aid in identifying potential hazards and hazardous events that may influence water safety.

Both solar reactors were able to significantly reduce the level of microbial contamination in the tank water samples for all microbial indicators evaluated, to below the drinking water guideline limits [with the exception of HPC in the Prototype I treated samples (43%)], through the use of an 8 hour solar radiation exposure. Although HPC exceeding the DWAF (1996) drinking water guideline limit were recorded in 43% of the Prototype I treated samples, a mean 1.01 log reduction in heterotrophic bacteria was recorded for these samples, which would decrease the health risk associated with using the treated rainwater (in comparison to the utilisation of untreated rainwater). Based on national and international drinking water guidelines (which predominantly employs culture-based analysis), the large-volume batch solar reactor prototypes used in the current study may effectively treat rainwater to within drinking water standards and provide water to the inhabitants of rural areas and urban informal settlements in sub-Saharan Africa. Results from the EMA-qPCR and PMA-qPCR analysis however, indicated that *E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* spp. oocysts were reduced by 74.43% in both reactor prototypes. The discrepancy in the results obtained using culture- and molecular-based analyses highlights the limitations of solely using traditional culture-based analyses to monitor water treatment systems, as an over-estimation of treatment system efficiency may be obtained. Thus, results obtained using molecular-based assays may be more representative of the viable and intact community in the treated water source, and a more accurate indication of the health risk to the end-user may be calculated when this data set is employed in quantitative microbial risk assessment (QMRA). Current research by the WATERSPOUTT research consortium is thus aimed at applying QMRA to monitor the quality of the treated rainwater.

Conflicts of interests

The authors have no conflicts to declare.

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Table 1 Frequency of detection and mean concentrations (CFU/100 mL) of indicator organisms and target bacterial pathogens in the tank water samples collected from sites 1 and 2.

Organism	Site 1		Site 2	
	Tank 1 (*n* = 15)	Prototype I (*n* = 15)	Tank 2-FF (*n* = 18)	Prototype II (*n* = 18)
E. coli	67% (6)	BDL	51% (3)	BDL
Total coliforms	100% (1.5×10^4)	27% (42)	100% (1.0×10^3)	11% (2)
Enterococci	20% (3.0×10^3)	BDL	28% (2.2×10^3)	BDL
Faecal coliforms	73% (1.2×10^4)	BDL	22% (1.1×10^3)	BDL
Heterotrophic bacteria	100% (3.5×10^5)	50% (1.8×10^4)	100% (6.9×10^4)	86% (6.5×10^3)
Klebsiella spp.	100% (1.9×10^4)	BDL	17% (8.0×10^2)	BDL
Pseudomonas spp.	ND	ND	ND	ND
Salmonella spp.	60% (6.3×10^3)	BDL	6% (1.0×10^3)	BDL
Coliphages (PFU/mL)	ND	ND	ND	ND

BDL – below detection limit; ND – not detected; PFU – plaque forming units

Figure 1

Fig. 1. (A) The Prototype I (140 L) solar reactor installed at Site 1. **(B)** The Prototype II (88 L) solar reactor installed at Site 2. The red arrow indicates the first-flush diverter which was connected to Tank 2-FF.

Figure 2
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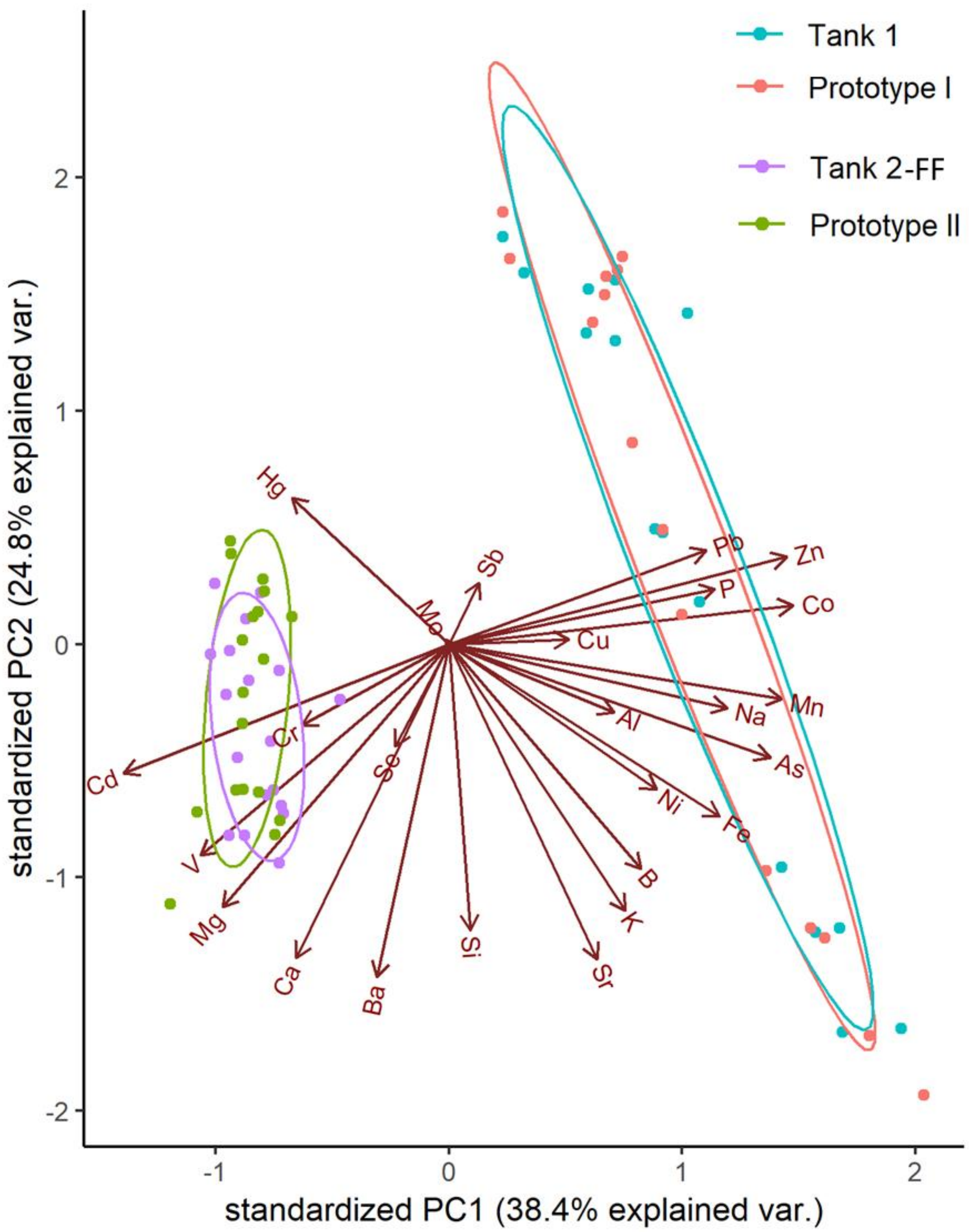

Fig. 2. Principle component analysis of the cations affecting the tank water quality for site 1 (Tank 1 and Prototype I) and 2 (Tank 2-FF and Prototype II). The directionality of the arrows indicate the correlation (same = positive; opposite = negative) between the different variables and illustrate the predominant variables best describing the collected tank water samples.

Figure 3
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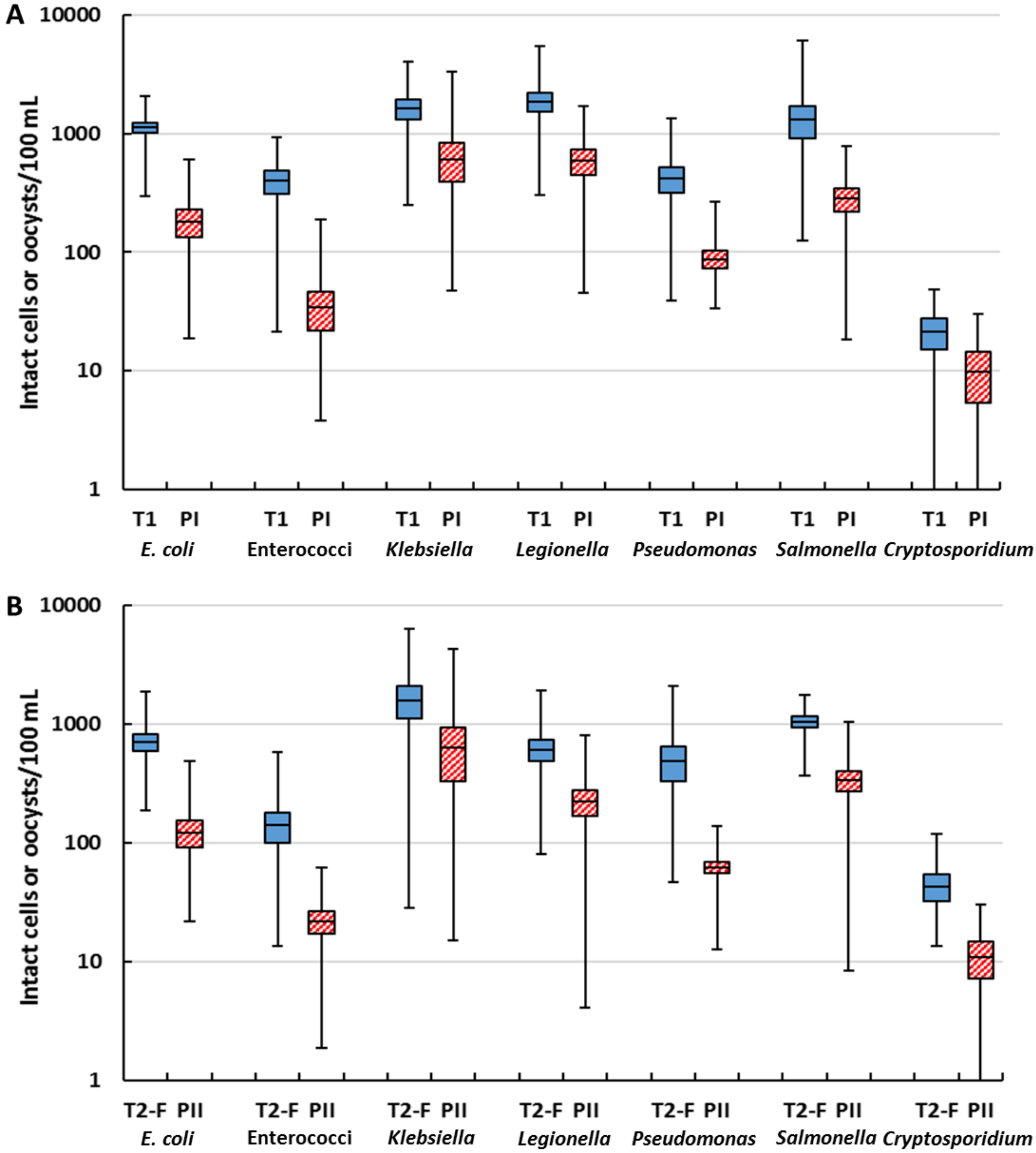

Fig. 3. Box and whiskers plot illustrating the distribution of the intact cells or oocysts/100 mL recorded for each of the target organisms using EMA-qPCR (*E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp.) and PMA-qPCR (*Cryptosporidium* spp. oocysts) in the untreated (T1 and T2-FF; solid blue box) and treated (PI and PII; dashed red box) tank water samples collected from **(A)** site 1 and **(B)** site 2. The whiskers at the end of each box indicate the minimum and maximum values, while the box is defined by the lower and upper quartiles and the mean value.

***conflict of Interest Statement**

Declaration of interests and Conflict of Interest Statement

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors have no conflict of interest to declare.

Thank you for your time and cooperation.

Yours sincerely

Wesaal Khan
Corresponding author: Stellenbosch University